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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> A01H 1/04, 5/00, C12N 5/04, 15/29, 15/64, 15/82, C12Q 1/00	<b>A1</b>	<b>(11) International Publication Number:</b> WO 96/32007 <b>(43) International Publication Date:</b> 17 October 1996 (17.10.96)
<b>(21) International Application Number:</b> PCT/US96/05272 <b>(22) International Filing Date:</b> 15 April 1996 (15.04.96)  <b>(30) Priority Data:</b> 113373 13 April 1995 (13.04.95) IL  <b>(71) Applicants (for all designated States except US):</b> YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; The Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). YISSUM RESEARCH AND DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; 46 Jabotinsky Street, P.O. Box 4279, 91042 Jerusalem (IL).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> FLUHR, Robert [US/IL]; 19/3 Ben-Yehuda Street, 76301 Rehovot (IL). ESHED, Yuval [IL/IL]; 34 Hador Street, 81523 Yavne (IL). ORI, Naomi [IL/IL]; 39 Hador Street, 81523 Yavne (IL). PARAN, Ilan [IL/IL]; 6 Harakefet Street, 99797 Carmel Yossef (IL). ZAMIR, Daniel [IL/IL]; 10 Derech Eretz, 70700 Gedera (IL).  <b>(74) Agent:</b> BROWDY, Roger, L.; Browdy and Neimark, Suite 300, 419 Seventh Street N.W., Washington, DC 20004 (US).		<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> TRANSGENIC TOMATO PLANTS CONTAINING A FUSARIUM RESITANCE GENE  <b>(57) Abstract</b>  The invention provides genes from the I2 Fusarium resistance locus of tomato belonging to a multigene family herein designated I2C. The DNA molecules of the invention are useful as a tomato resistance gene to plant vascular diseases caused by Fusarium pathogens, particularly <i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i> race 2, or as probes for breeding Fusarium-resistant tomato lines or for screening of new diseases in plants of the Solanaceae family. Further provided are Fusarium-resistant tomato lines transformed by an I2C resistance gene of the invention.		

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## Transgenic Tomato Plants Containing a Fusarium Resistance Gene

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### FIELD OF THE INVENTION

The present invention relates to genes from the *I2* Fusarium resistance locus of tomato belonging to a multigene family herein designated I2C, useful either as a tomato resistance gene to plant vascular diseases caused by Fusarium pathogens, or as probes for breeding Fusarium resistant tomatoes or for screening of new diseases in related plants of the Solanaceae family, and to transformed plants, particularly Fusarium resistant tomatoes.

### BACKGROUND OF THE INVENTION

Resistance to pathogens is thought to involve a specific recognition between a resistant plant and the pathogen, which triggers a set of responses that act to confine the pathogen. The specificity of this process is considered to involve a recognition between the products of a plant resistance (R) gene and a cognate pathogen avirulence gene (Dangl, 1995; Staskawicz et al., 1995). The characterization of resistance genes is of major importance for elucidating the initiation of the cascade of events that leads to specific resistance responses, as well as for more efficient introduction of resistance to pathogens into important crops.

Several resistance genes have been cloned recently by positional cloning or by transposon tagging. These genes include: the *HMI* gene of maize (Johal and Briggs, 1992), the *Pto* gene of tomato (Martin et al., 1993), the *Cf-9* gene of tomato (Jones et al., 1994), the *RPS2* (Bent et al., 1994; Mindrinos et al., 1994) and the *RPMI* (Grant, 1995) genes from *Arabidopsis*, the *N* gene from tobacco (Whitham et al., 1994), and the *L6*

gene from flax (Ellis et al., 1995; Lawrence et al, 1995). These resistance genes show diverse biological characteristics. The *HMI* gene is the only example to date where the gene product acts directly to inactivate a component of the pathogen attack, or a compatibility factor (Briggs and Johal, 1994). The other genes belong to a different genetic category, that of incompatibility (or gene for gene) interaction, based on the recognition by the resistance gene product of an avirulence (or incompatibility) component of the pathogen, which does not necessarily participate in the compatibility or in the infection processes (Briggs and Johal, 1994). These genes are all involved in resistance processes characterized by hypersensitive response (HR). In spite of their origin from different plant species, and their divergent specificity to viral, fungal or bacterial pathogens, a group of these R genes share several structural features. A nucleotide-binding domain (P-loop) and five additional amino-acid stretches of unknown function are conserved in their N-terminal region. A region of leucine-rich repeats (LRR) is present in their C terminus, though the consensus sequence and the length of the repeats are different among them. LRR were shown to be involved in protein-protein interactions in other proteins (Kobe and Deisenhofer, 1994; Kobe and Deisenhofer, 1995), and may have similar role in resistance genes. The *N* gene, the *L6* gene and the *Cf-9* gene were shown to belong to large gene families, partially clustered with the resistance gene. The detailed genomic distribution of these multigene families is yet unknown.

The soil-born fungus *Fusarium oxysporum* is the causative agent of severe wilt diseases in a large variety of plant species world-wide. It is an imperfect fungus for which no sexual cycle is known. The tomato-specific pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*F.o.l*) causes the disease Fusarium wilt. The fungus penetrates the vascular system of roots from both resistant and susceptible varieties, mainly through wounds. During a compatible interaction, which leads to disease, the fungus proceeds through the vascular system which eventually collapses. This leads to wilt and often to death of the plant. During an incompatible interaction, resulting in resistance, the fungus is confined to the lower part of the roots, and further symptoms do not develop. Several mechanisms, not including HR, were suggested to be involved in this resistance. They include: the production of inhibitory secondary metabolites, and structural barriers such as vascular gelation, callose deposition, and abnormal membrane outgrowths of vascular

parenchyma cells, termed tyloses. Most of these processes, thought to be involved in resistance to vascular diseases, are detectable also in compatible interactions, though to a lesser extent. Therefore the exact sequence of events that leads to resistance is still unknown.

5 Three races of *F.o.I.* and their cognate R genes have been identified in tomato. The classification of different *F.o.I.* isolates into races does not correlate with their general genetic resemblance, as established by restriction fragment length polymorphism (RFLP) analysis and distribution into vegetative compatibility groups (VCG; Elias et al., 1993). The *I* locus, introgressed from *L. pimpinellifolium*, confers resistance to *F.o.I.* race  
10 1, and is located on the short arm of chromosome 11, between the RFLP markers *TG523* and *CP58* (Eshed and Ori, unpublished). The *I3* locus from chromosome 7 of *L. pennellii* confers resistance to races 1, 2 and 3 of *F.o.I.* (Bournival et al., 1990). This locus appears to be composed of three separate but linked genes (Scott and Jones, 1991). The *I2* locus, introgressed from *L. pimpinellifolium*, confers resistance to race 2 of the pathogen. We  
15 previously mapped *I2* to the long arm of chromosome 11, between the RFLP markers *TG105* and *TG36*, very close to *TG105* (Segal et al., 1992; Ori et al., 1994). In previous studies we utilized recombinant inbred (RI) lines for mapping *I2* (Ori et al., 1994). However this population turned to be problematic for mapping of this region because of a very high recombination rate, including double recombinations, especially in the region  
20 of *I2*.

### SUMMARY OF THE INVENTION

It has now been found in accordance with the present invention that high resolution genetic and physical mapping of the *I2* region, using a large and conclusive F2  
25 population (3200 meiotic gametes), show complete cosegregation between *I2* and a cluster of genes on chromosome 11 belonging to a new multigene family, herein designated *I2C*.

Additional multigene family members are dispersed between four different loci, on three different chromosomes, either in clusters or as single genes. Two *I2C* genomic  
30 clones were isolated from the locus completely linked to *I2* and sequenced, and were herein designated *I2C-1* and *I2C-2*. Their sequences show striking structural similarity with a group of recently isolated resistance (R) genes, which includes the above-

mentioned *RPS2* and the *RPM1* genes from *Arabidopsis*, the *N* gene from tobacco and the *L6* gene from flax. These genes confer resistances to specific pathogens of viral, bacterial and fungal origin, and share common features. They contain a conserved nucleotide binding domain, termed P-loop, in their N terminus, and five other conserved domains of unknown function. At least half of their C terminus is composed of leucine rich repeats (LRR).

A few partial cDNA clones from the I2C family were further examined, such as the herein designated I2C-3 and I2C-4 cDNA clones, and show that family members differ from each other mainly by insertions or deletions.

10 The deduced amino acid sequence encoded by members of this gene family reveals a region of LRRs, as well as a P-loop and other motifs in common with the above-mentioned recently characterized plant resistance genes.

Thus, in one aspect, the present invention provides a DNA molecule selected from the group comprising:

- 15 (i) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-1 (SEQ. ID. NO.:1);
- (ii) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-2 (SEQ. ID. NO.:2);
- (iii) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-3 (SEQ. ID. NO.:3);
- 20 (iv) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-4 (SEQ. ID. NO.:4);
- (v) a DNA molecule characterized by containing a coding sequence representing at least 60% similarity with the encoded open reading frame in the DNA sequence of at least one of the DNA molecules (i) and (ii);
- 25 (vi) a DNA molecule capable of hybridization with any one of the DNA molecules (i)- (v) under moderately stringent conditions;
- (vii) a DNA molecule that differs, by insertion, deletion or as a result of the degenerative nature of the genetic code, from the DNA sequences (i)-(vi); and
- 30 (viii) a fragment of any of the DNA molecules (i)- (vii).

The DNA molecule defined in (v) above contains preferably a coding sequence representing 70-80% similarity with the encoded open reading frame in the DNA

sequence of at least one of the DNA molecules (i) and (ii). The moderately stringent conditions required in (vi) above are such as those conditions described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. edition, Cold Spring Harbor Laboratory Press, New York, 1989.

5 One of the members of the multigene family I2C consisting of a DNA molecule as defined in (i)-(vii) above will confer resistance to *Fusarium oxysporum* f.sp *lycopersici* race 2 in tomato plants. In another aspect, the invention relates to a gene construct comprising such DNA as a genomic clone including regulatory sequences that flank the coding region thereof, and to a cosmid, into which said gene construct has been  
10 subcloned, for direct transformation of tomato plants.

In another embodiment, a DNA molecule according to the invention may be subcloned into a plant transformation vector under the control of regulatory elements capable of enabling the expression of said DNA molecule in plant cells. Said DNA regulatory sequences comprise, for example, a plant promoter, a DNA sequence that  
15 enhances translation of the mRNA transcribed from said DNA molecule and a polyadenylation/terminator sequence.

In a further embodiment the invention provides a tomato cell line or a tomato plant line transformed with a cosmid or with an expression vector of the invention, and to tomato plants regenerated from said transformed cells

20 In another aspect of the invention, a DNA molecule or fragment thereof as defined in (i)-(viii) above may be used as a direct RFLP probe employing standard protocols for breeding tomatoes resistant to *Fusarium oxysporum* f.sp *lycopersici* race 2, or to examine the homologous multigene family in related plants of the Solanaceae family, e.g. potato, pepper, petunia, eggplant, preferably plants which have colinear  
25 genomic maps with tomatoes, for finding new species-specific disease linkages with said probes. The thus bred tomato plants and related plants of the Solanaceae family are also encompassed by the present invention.

Thus the invention provides a method of selective breeding of *Fusarium* resistant tomatoes employing a DNA molecule according to the invention as a direct restriction  
30 fragment length polymorphism (RFLP) probe, which comprises:

(i) marking said DNA molecule with a suitable marker; and

(ii) reacting said probe of (i) with DNA extract of a tomato plant under hybridization conditions;

thus obtaining restriction-length polymorphism that is indicative of a resistance-type gene, which facilitates the selection of progeny that contains said resistance gene.

The invention further provides a method of screening new diseases in plants of the Solanaceae family employing a DNA molecule according to the invention as a direct RFLP probe which comprises:

(i) marking said DNA molecule with a suitable marker; and

(ii) reacting said probe of (i) with DNA extracts from said Solanaceae species,

thus identifying the homologous gene family in those species which can be linked to known resistance genes in those species.

## **DESCRIPTION OF THE DRAWINGS**

Figs. 1A-C depict genetic and physical maps of the tomato gene *I2* region. (1A) Genetic linkage map of chromosome 11, adopted from Eshed et al. (1995). The *I* and *I2* Fusarium resistance loci were positioned according to Eshed (unpublished) and Segal et al. (1992), respectively; (1B) High resolution mapping of the genetic region spanning RFLP markers *TG105A* and *TG36*, as revealed from analysis of 1600 F2 and F3 individuals; (1C) Physical map of YAC 340-63, with relevant markers indicated. The total length of YAC 340-63 is 350 kb.

Fig. 2 shows Southern blot analysis of genomic tomato DNA of resistant and susceptible parental types and of the fixed recombinant F2 plant BR 30(5). DNA samples were digested with *TaqI*, and the blot hybridized with *SL8* probe. R and S indicate *F.o.I* race 2 resistant and susceptible individuals, respectively. R lanes, parental types which are a nonrecombinant resistant type F2 individual from the F2 population initiated from Br5577; S lanes, parental lines which are the sensitive tomato inbreds *L. esculentum* var. M82 and *L. esculentum* var. S-365. BR 30(5) is the single recombinant identified within the *SL8* cluster, from the entire F2 population. TR1-TR8 indicate resistant-type polymorphic bands, as established by examination of this and additional gels. Sizes in kb are indicated on the right.



Figs. 3A-B show distribution of *SL8*-homologues in the tomato genome. (1A) Linkage maps of chromosomes 8, 9 and 11. The linkage maps were adopted from Eshed et al. (1995). The relevant introgressed regions of the ILs are illustrated on the right of each tomato linkage map (solid lines). Asterisks indicate approximate map positions of known disease resistance genes. The mapped positions of the *SL8* loci are indicated; (1B) Southern blot of *TaqI* digested DNA of representative ILs. *L. pennellii* fragments in the blot are designated A, B, C, D and E according to their genomic location, as indicated in panel A.

Fig. 4 depicts the nucleotide sequence and deduced amino acid sequence of the clone herein designated I2C-1 [SEQ. ID. NOS:1 and 5, respectively]. The first translated nucleotide is no. 1. Sequences conserved between resistance genes are double underlined. Leucine Rich Repeats (LRRs) region and other AA repeats are single underlined. A putative Leucine-Zipper domain is underlined with dots.

Fig. 5 depicts the nucleotide sequence and deduced amino acid sequence of the clone herein designated I2C-2 [SEQ. ID. NOS:2 and 6, respectively]. The first translated nucleotide is no. 1. Sequences conserved between resistance genes are double underlined.

Fig. 6 depicts the partial nucleotide sequence of the 3' of the cDNA clone herein designated I2C-3. [SEQ. ID. NO:3]

Fig. 7 depicts the partial nucleotide sequence of the 3' of the cDNA clone herein designated I2C-4. [SEQ. ID. NO:4]

Fig. 8 shows comparison of the deduced amino acid sequences of the genomic clones I2C-1, I2C-2 [SEQ. ID. NOS:5 and 6] and of the resistance genes RPS2 and RMP1 from *Arabidopsis*, N from tobacco and L6 from flax (Bent et al., 1994; Dangl, 1995; Grant, 1995; Jones et al., 1994; Mindrinos et al., 1994; Lawrence et al., 1995; Whitham et al., 1994) [SEQ. ID. NOS:7, 8 and 9]. Residues numbers are from the first translated methionine of each sequence. Consensus sequence in the N terminal region is indicated only when minimum number of gaps was needed for alignment of at least 5 out of 6 residues. Symbols are: con., consensus sequence; a, aliphatic residue; - and +, negatively or positively charged residues, respectively. Boxes containing stretches of conserved residues are indicated by a line above the sequences, and numbered from I to VI.

Fig. 9 shows alignment of the leucine reach repeats (LRR) of I2C-1 [SEQ. ID. NO:5], and their consensus sequence. (Top) Alignment in the region from residues 558 to 1220 of I2C-1 where alignment to consensus sequence is optimized; (Bottom) A comparison of the consensus sequences of the I2C-1 LRR with those of the resistance genes RPS2, N and Cf-9, and the T-LR SAG expression site associated leucine rich protein from *Trypanosoma brucei*.  $\alpha$  represents an aliphatic residue.

Fig. 10 shows comparison of the 3' end of four I2C family members. I2C-1 and I2C-2 are the deduced amino acid sequences [SEQ. ID. NOS:5 and 6] as in Fig. 8. I2C-3 and I2C-4 are the deduced amino acid sequences [SEQ. ID. NOS:10 and 11] derived from partial cDNA clones from a  $\lambda$ gt 10 library. The sequence of I2C-4 is a chimera between three ORFs, originally separated by one base insertions which caused two frame shifts. The junctions where separated ORFs were combined are indicated by arrows. Con. indicates consensus and is shown when a residue is present in all 4 sequences. Numbers are from the first methionine in sequences I2C-1 and I2C-2 [SEQ. ID. NOS:5, 6] and from the first residue of the available sequence for I2C-3 and I2C-4 [SEQ. ID. NOS:10 and 11]. Brackets indicate a repeat unit of 23 amino acids which appears in variable copy number in the 3' end of the cDNA clones.

Fig. 11 depicts cosmids 12-134 and 12-150, which contain the genes I2C-1 and I2C-2, respectively, in the BamHI site of cosmid TDNA 04541.

Fig. 12 shows sense constructs from the I2C-134 cosmid, which contains the genomic clone I2C-1 prepared in the PGA492 binary vector.

Fig. 13 shows sense (2-1 and 5-1) and antisense (6-3 and 31-17) constructs comprising the genomic clone I2C-1 or the cDNA clone I2C-3 for transformation of *Fusarium* resistant plants.

## **DETAILED DESCRIPTION OF THE INVENTION**

The invention relates to a multigene family, I2C, which is dispersed on three different chromosomes of the tomato genome. Two lines of evidence suggest that a member of this gene family is the *I2 Fusarium* resistance gene. The first is the complete cosegregation of some of the genes from this family with the *I2* gene; the second is the striking structural similarity between members of this family and a group of recently isolated plant resistance genes (Bent et al., 1994; Dangl, 1995; Grant et al., 1995; Jones

et al., 1994; Mindrinos et al., 1994; Lawrence et al., 1995; Whitham et al., 1994). In addition, *I2C* genes from the *SL8D* locus of the family, which maps to *I2*, were shown here to be very highly polymorphic between *F.o.l.* resistant and susceptible varieties. In a similar fashion, the *Pto* resistance gene was also shown to be a gene family highly polymorphic between bacterial speck resistant and sensitive varieties (Martin et al., 1993). Which of the family members are responsible for resistance can be determined by extensive complementation tests with all members of the *SL8D* cluster.

The *I2C* gene family contains a few motifs that have been identified in plant resistance genes. The N terminus contains a P-loop and 5 additional conserved boxes of unknown function. Different classes of P-loop motifs are common to many but not all nucleotide binding proteins (Saraste et al., 1990). The consensus of this motif varies significantly between different classes of nucleotide binding proteins, but is highly conserved within each class. The consensus sequence GMGGaGKTT, where a designates an aliphatic amino acid, is highly conserved among the P-loops of the genes *I2C-1*, *I2C-2*, *RPS2*, *RPM1*, *N* and *L6*. No other protein in the gene bank were found to contain this consensus sequence. In contrast to the *Cf-9* gene, and perhaps the *RPS2* gene, the *I2C-1* deduced protein sequence does not predict any membrane-spanning domain. Residues 623 to 645 of *I2C-1*, included in the LRR region, fit the consensus for a leucine zipper (Busch and Sassone-Corsi, 1990). This motif is considered to be involved in dimerization of DNA binding proteins. However, sequences that fit this consensus are abundant in the databanks, and the existence of this consensus does not necessarily imply a function.

In common with recently isolated plant resistance genes, the C-terminal parts of the *I2C* genes are composed of leucine rich repeats. The LRR consensus comprising 23 amino acids, together with a lack of a membrane spanning domain in the gene, are consistent with an intracellular location of this gene family (Jones et al., 1994). The LRRs of four members of the *I2C* family show high homology to each other and differ from each other mainly by insertions or deletions. This may be indicative of evolutionary processes, and hint at mechanisms that generate new diversity of LRR. Interestingly, one of the cDNA analyzed, *I2C-4*, contains stop codons within the LRR region, and therefore may result in a truncated protein. This is reminiscent of phenomena described for the *N* and the *L6* genes, where truncated transcripts were described, apparently arising from alternative splicing. In the case of *I2C-4* this mechanism is less likely, since the genes

analysed thus far from the family appear to lack introns. The *I2C* LRR-region consensus is homologous to the trypanosome variable surface glycoprotein (VSG) expression site associated gene *T-LR*. This gene is thought to be involved in the regulation of adenylate cyclase function (Ross et al., 1991; Smiley et al., 1990). LRR were described recently in  
5 many proteins, and may be involved in protein-protein interactions (Colicelli et al., 1990; Kobe and Deisenhofer, 1994). Thus, the LRR region may be responsible for specificity of interaction, either with a protein component from the pathogen, or with downstream factors involved in signal transduction. The crystal structure of an interaction of an LRR containing protein, an RNAase inhibitor (RI), with RNAase, was recently described  
10 (Kobe and Deisenhofer, 1995). The RI contains a horse-shoe like structure in which individual, 28-29 residues long repeats constitute b-a hairpin units which are aligned parallel to a common axis (Kobe and Deisenhofer, 1993). However, the LRR consensus of *I2C* and of the other plant resistance genes differ from that of RI. In addition, the repeat length of *I2C* varies between 19 and 32 amino acids per repeat. Similar variation  
15 in repeat length can be observed in other R genes, which may imply a less organized or different structure than that found for RI.

Using novel tomato genetic populations (Eshed et al., 1992; Eshed and Zamir, 1994; Eshed and Zamir, 1995), all members of the *I2C* family have been mapped according to the present invention. The *I2C* genes are distributed to five locations in the  
20 genome, two of which are clusters of several genes, both on chromosome 11. Some of the recently isolated resistance genes were also shown to be members of large, clustered, gene families, but complete mapping data for them is lacking. The complex pattern of distribution of *I2C* is remindfull of the case of *L* and the *M* rust resistance genes of flax (Ellis et al., 1995). In that case, *L* appears to be a single multiallelic gene, whereas  
25 homologous sequences map to a more complex *M* locus containing a gene cluster. Both loci may contain resistance genes specific for different races of the same pathogen, or to different pathogens. It is interesting to note in this respect, that the *I2C* copy from chromosome 9 (*SL8B*) maps with a resolution of 5 cM to both the *Tm-2a* TMV resistance gene (Young et al., 1988), and the *Frl* *Fusarium oxysporum* f. sp. *radicis lycopersici*  
30 (*F.o.r.l.*; Laterrot and Moretti, 1995). The *I2C* cluster *SL8C* maps in the vicinity of the *Sm* *Stemphylium* resistance gene, with a resolution of 10 cM (Behare et al., 1991). However, no member of the *I2C* family maps to the *I* locus on the short arm of

chromosome 11, or the *I3* locus in chromosome 7 (Bournival et al., 1990; Scott and Jones, 1991), which confers resistance to races 1, 2 and 3 of *F.o.f.*

Considering that a member of the *I2C* family encodes for the *I2* resistance gene, the present invention shows commonalities between a wilt disease resistance gene and other disease resistance genes. Despite the lack of HR in vascular disease resistance, the *I2C* family belongs to the superclass of resistance genes described for leaf HR. This raises questions concerning the role of the various functional domains of R genes in upstream and downstream events that result in different types of resistance mechanisms.

For transformation of tomato plants, a genomic *I2C* clone according to the invention may be subcloned into any suitable cosmid, such as cosmid TDNA 04541 (Jones et al., 1992). Such constructs contain more than a few kb of genomic DNA upstream and downstream from the gene coding region sufficient for regulated expression. These constructs can be used for direct DNA transfer into plant cells by electroporation (Dekeyser et al., 1990); by polyethyleneglycol (PEG) precipitation (Hayashimoto et al., 1990), by ballistic bombardment (Gordon-Kahn et al., 1990), or by *Agrobacterium*-mediated transformation (Jones et al., 1992).

Other engineered constructs according to the invention comprise a DNA molecule of the invention and DNA regulatory elements enabling the expression of said DNA molecule in plant cells. Said DNA regulatory sequences comprise, for example, a plant promoter, a DNA sequence that enhances translation of the mRNA transcribed from said DNA molecule and a polyadenylation/terminator sequence.

The plant promoter used in the invention is selected from tissue specific and non-tissue specific plant promoters of different kinds, derived from both mono- and dicotyledoneous plants. The preferred promoter is the commercially available cauliflower mosaic virus (CaMV) 35S promoter that is generally expressed in most, if not all, plant tissues, including vascular tissues. Another example of promoter expressed in vascular tissues that can be used in the invention is PRB-1b (Eyal et al., 1993).

The promoter is to be found in the 5' region of the gene. At the 3' end of the promoter, a short DNA sequence for 5' mRNA non-translated sequence may be added to enhance translation of the mRNA transcribed from the gene, such as the omega sequence derived from the coat protein gene of the tobacco mosaic virus (Gallie et al., 1987).

Downstream at the 3' end of the resistance gene DNA coding sequence a terminator DNA sequence containing the 3' transcription termination and polyadenylation signal of the mRNA from the resistance gene is installed. Terminator DNA sequences comprised within the 3' flanking DNA sequences of any cloned genes  
5 can be used, such as the 3' untranslated sequence of the octopine synthase gene of the Ti plasmid of *Agrobacterium tumefaciens* (Greve et al., 1983), or more preferably the 3' untranslated sequence of the nopaline synthase gene (Depicker et al., 1982).

The gene constructs of the invention can be subcloned into expression vectors, such as the Ti plasmids of *Agrobacterium tumefaciens*, the preferred plasmid being the  
10 pGA492 binary vector (An., 1986).

The expression vector comprising the resistance gene is then introduced into plant cells by a transformation protocol capable of transferring DNA to dicotyledoneous plant cells, preferably by infection of plant cells with *Agrobacterium tumefaciens* using the leaf-disk protocol (Horsch et al., 1985). For this purpose, tomato leaf explants are  
15 infected and the transformed tomato cells are cultured on a suitable medium, preferably a selectable growth medium. Tomato plants can then be regenerated from the resulting callus. Tissue cultures of transformed tomato cells are propagated to regenerate differentiated transformed whole plants. Transgenic plants are thereby obtained whose cells incorporate a *Fusarium* resistance gene in their genome, said gene being expressible  
20 in the cells. Seeds from the regenerated transgenic plants can be collected for future use. Transformed plants that are resistant to *Fusarium oxysporum* f.sp. *lycopersici* race 2 can be selected by incorporating a selectable marker such as resistance to kanamycin.

The DNA molecules of the invention can further be used as markers in selective plant breeding as direct RFLP probe as exemplified in Examples 2 and 3 and Figs. 2 and  
25 3 herein, or small fragments thereof can be used for PCR-based technology in marker assisted breeding (review by Tanksley et al., 1995).

In the RFLP probe technology, the DNA from different varieties of tomatoes or related Solanaceae plants is digested by restriction enzymes and fractionated on an agarose gel. Digests are chosen such that the gene of interest will be polymorphic. The  
30 DNA fragments are transferred to a nitrocellulose or other similar blotting agent and hybridized to the I2C probe of the invention, preferably under conditions of 6xSSC, 0.5% SDS, at 65°C. The blots are washed, preferably at 2xSSC at room temperature, and

subjected to autoradiography. Further washings at higher temperatures and lower SSC concentrations can be carried out for higher stringency.

The individual DNAs in the population that show the polymorphic signature of the resistance gene can then be further used in a breeding program for the desired traits.

5       An advantage of using these DNA sequences as direct RFLP probes in selective plant breeding is that the presence of disease resistance in plants can then be examined without using phytopathological methods. In addition, by using a direct or tightly-linked DNA marker as probe (based on RFLP or PCR-based technologies), it is possible to select for the desirable trait, i.e. resistance gene without accompanying genetic drag, i.e.  
10       transfer of the desired trait by breeding without incorporation of flanking unwanted traits.

      The DNA molecules of the invention can further be used as probes to identify homologous multigene families conferring resistance to different diseases in related plants of the Solanaceae family, such as those which have colinear genomic maps with  
15       tomatoes, e.g. potato, eggplant, pepper, petunia and the like, for example, by using a marker of the I2C-1 family as a DNA probe to clone the related gene family from said related Solanaceae species, and then using that clone or I2C members directly as DNA probes to analyze genetic lines of those species for RFLP linkage to resistances in said species.

20       In the PCR-technology, a preferred approach uses specific oligonucleotides synthesized according to the sequences of selected regions of the I2C family such that the fragment generated in a PCR reaction will yield a polymorphic band for the resistance trait either by being specific for the resistance-type gene and therefore yielding a null PCR reaction in sensitive plants, or by yielding PCR fragments of different size or  
25       different restriction patterns upon using sensitive or resistant sources of the DNA.

      The invention now will be illustrated according to the following non-limiting Examples and the drawings herein.

### **EXAMPLES**

30       In the Examples, the following Materials and Methods are used.

## MATERIALS AND METHODS

### (a) Plant material and genetic mapping

Four *L. esculentum* segregating F2 populations were used for genetic mapping. In each case, an initial cross was made between a parent resistant to *F.o.l* race 2 (R), and a susceptible parent (S). The resistant and susceptible parents for the first 3 populations were, respectively, c.v. Motelle and c.v. Money Maker; c.v. Mogeor and c.v. Vendor; and c.v. Motelle and LA 1113 (chromosome 11 marker stock, kindly provided by Dr. C.M. Rick (UC Davis). The fourth population was initiated from the commercial hybrid line Br5577 (AB Seeds, Ness Ziona, Israel), as F1. The results obtained from the 4 populations were pooled.

### (b) Genomic and cDNA libraries, plasmids and probes.

YAC8<sup>+</sup> (YAC 340-63, Cornell collection), which contains the RFLP marker *TG105A*, was generated from the *F.o.l* race 2 resistant tomato line Rio Grande - PtoR, and cloned in the vector pYAC 4 (Martin et al., 1992). Probes from YAC 340-63 that were used for the genetic and physical mapping are as follows. D2 is from a genomic lambda library of the tomato line VFNT cherry, selected during chromosome walking from *TG105*. SL8, SR8 and 6-16, are subclones of IEMBL3 clones from a library of the yeast line that contains YAC 340-63. D14 is a cDNA clone selected by YAC 340-63 from a cDNA library from roots of the *F.o.l* race 2-resistant tomato *L. esculentum* c.v. Mogeor. Additional clones, previously described (Ori et al, 1994), were not polymorphic or not informative in these populations.

cDNA clones which represent members of the I2C family were isolated from three different cDNA libraries. The cDNA libraries were all constructed in  $\lambda$ gt10, from roots or leaves of resistant-type *L. esculentum*. Positive clones were equally abundant in the three different libraries. While large (>3 kb sizes) clones have been previously isolated from these libraries, all the SL8 clones were partial and contained only the 3' end of the genes. Cosmid clones were isolated from a genomic library of the *F.o.l* race 2 resistant variety *L. esculentum* var. Mogeor, constructed in the cosmid TDNA 04541 (Jones et al., 1992).



### (c) Sequence analysis

Cosmid clones were either subcloned into the Bluescript plasmid or sequenced directly. The cDNA clones were subcloned into the Bluescript vector and then sequenced. Sequencing was performed with an automated Applied Biosystems  
5 Sequencer. Sequence analysis was performed using the sequence analysis software package of the 'University Wisconsin, Genetics Computer Group' (Devereux et al, 1984).

### (d) Physical mapping

Yeast DNA for pulse field gel electrophoresis analysis was digested with limiting  
10 amounts of the restriction enzymes MluI, XhoI and Sall, to obtain successive partial digestions. The digests were fractionated on counter clamped homogeneous electric field (CHEF) gels (BioRad), blotted and hybridized with probes. The maximal distance between a pair of markers was estimated according to the smallest partial band that contained both markers. Additionally, the DNA was digested with the rare cutters SgrAI  
15 and PmeI.

### (e) Genetic mapping

RFLP analysis and *F.o.I.* inoculation were performed as previously described (Segal et al., 1992). F2 plants (1200) were screened for recombinants between *TG105* and *TG36*. When necessary, F3 seedlings from the recombinant plants were screened in  
20 order to fix the recombination to a homozygous state. An additional 400 F3 plants were screened, and more recombinants were identified in the region. All the recombinants were analyzed for the different RFLP markers and for Fusarium resistance.

### Example 1. High resolution mapping of I2

25 The *I2* Fusarium resistance gene was previously mapped to chromosome 11, between RFLP markers *TG105A* and *TG36*, 0.4 centimorgan from *TG105A* (Figure 1A, and Segal et al., 1992). To obtain higher resolution mapping of *I2*, we generated new markers in the region of *TG105A*, by chromosome walking from *TG105A* on lambda clones, and by subcloning a 350 kb YAC clone, YAC 340-63, that hybridized to  
30 *TG105A*. Pulse Field Gel Electrophoresis (PFGE) of YAC 340-63 was used to physically position genetically informative markers, as shown in Figure 1C. In order to localize the position of *I2* relative to the new markers, a segregating population of 1600 plants (F2

and F3) was screened for recombinations between *TG105A* and *TG36*, and 57 recombination events were detected. The recombinant plants were then tested for *F.o.l* race 2 resistance, and for RFLP markers located between *TG105A* and *TG36*. According to the resulting map (Figure 1B), *I2* maps to the multi-copy marker *SL8D*, which represents the edge of YAC 340-63 (Figure 1C) and lies genetically between markers 6-16 and *TG36*, 0.23 cM from 6-16 and 1.3 cM from *TG36*. In previous studies we utilized recombinant inbred (RI) lines for mapping of *I2* (Ori et al, 1994). Interestingly, in spite of some inconsistencies in linearity of markers between the RI and the F2 population described here, *SL8D* completely cosegregated with *I2* also when mapped using the RI lines (data not shown). The *SL8* marker showed a remarkably high rate of polymorphism between *F.o.l* race 2 resistant and susceptible lines. In comparison, flanking markers showed a much lower degree of polymorphism, as judged by the paucity of restriction digests that yielded polymorphic bands.

**Example 2. *SL8* is a member of a gene family cosegregating with *I2***

The complete genetic cosegregation of *SL8D* with the *I2* resistance gene and the unique level of polymorphism between resistant and susceptible lines prompted us to further characterize the multicopy marker *SL8*. Sequence analysis revealed that *SL8* contains an open reading frame with similarity to a group of recently isolated resistance genes (see below). This suggested that *SL8* is a part of a gene that belongs to a family which includes the *I2* resistance gene. The gene of which the *SL8* probe was the 3' part was therefore designated *I2C'-1* (*I2* candidate 1).

We wished to further characterize the different *SL8* family members as RFLP markers, draw criteria to distinguish between them, and analyze their genomic distribution. A comparison of the *SL8* RFLP patterns of resistant and susceptible type lines, obtained with the restriction enzyme *TaqI*, is shown in Figure 2. Approximately 17 different *TaqI* bands hybridized to the *SL8* probe, and many of them were polymorphic between resistant and sensitive lines. Resistant-type bands, consistent among all tested lines, were designated TR1-TR8. The rest of the bands were either nonpolymorphic or polymorphic between the susceptible lines, because of the different parental origins. As several polymorphic *TaqI* bands were detected with the *SL8* probe, direct allelism cannot be established. In the entire F2 population, a single recombinant plant was identified

between all the polymorphic SL8-bands (BR 30(5) in Figure 2), which separated *SL8D* into two distinct loci, *SL8D-1* and *SL8D-2*. (Figure 1B). Except for this case, all polymorphic SL8 bands cosegregated. The recombinant individual BR30 (5) is sensitive to *F.o.l* race 2, and contains one resistant-type TaqI band, TR7, but lacks the others (TR1-6 and TR8). Similar additional southern blots and progeny tests of BR 30(5) confirmed these results (data not shown). Therefore, bands TR1-TR6 and TR8 appear to completely cosegregate with each other and with *I2*, and are all candidates for the resistance gene. However, the possibility of a recombination within the gene should also be considered. This could result in a sensitive plant containing a part of the resistance gene, and consequently a polymorphic band that belongs to the resistance gene. In addition, the possibility exists that a nonpolymorphic band represents the resistance gene. The latter is unlikely as will be shown below.

### Example 3. Genomic distribution of I2C

As only a subset of the *SL8* copies showed polymorphism between the parents of the F2 populations used for mapping, additional populations were incorporated to map all the SL8 fragments. The first is an introgression-lines (IL) population, in which single chromosome segments from *L. pennellii* were introgressed into a *L. esculentum* background. Both parental species of the IL population carry the susceptible allele at the *I2* locus (Eshed and Zamir, 1994). Figure 3A illustrates the genomic segments introgressed from chromosomes 8, 9 and 11 in the IL lines, which proved relevant for the *SL8* mapping. All *SL8* copies appear to be polymorphic between *L. esculentum* and *L. pennellii* (Figure 3B, lanes 1 and 2), a feature which facilitated their mapping. DNA digests from the IL lines were compared by southern blot hybridization with SL8 with that *L. esculentum* and *L. pennellii* (Figure 3B). *L. pennellii* bands that are contained in each IL, as well as their allelic *L. esculentum* bands that are absent from these lines, represent SL8 copies that originate from the region introgressed in the respective line. One *SL8* copy mapped to the short arm of chromosome 8 (*SL8A*), as one *L. pennellii*-type band is present, and one *L. esculentum*-type band is absent from IL 8-1. Similarly, one copy with weaker homology mapped near the centromere of chromosome 9 (*SL8B*; Figure 3A,B). More accurate location of these two copies was obtained using lines containing shorter introgressed segments of the region, derived by selection of

recombinants from the F2 of the original IL crossed back to *L. esculentum* var. M82 as illustrated in Figure 3A. The rest of the *SL8* copies mapped to the long arm of chromosome 11. Two of the introgression lines, IL 11-3 and IL 11-4, contain *L. pennellii* segments from the long arm of chromosome 11 (Figure 3A). By comparing these two lines, three genetically distinct groups of *SL8* family members could be identified on chromosome 11. The first (*SL8C*) maps to the region exclusively introgressed in IL 11-3, the second (*SL8D*) to the region of overlap between the IL 11-3 and IL 11-4, and the third (*SL8E*) to the region exclusively introgressed in IL 11-4. As previously established, the *I2* resistance gene maps to the region of overlap designated *SL8D*.

Higher resolution mapping of the chromosome 11-based *SL8* loci was accomplished using an F2 population of 150 plants, generated from an initial cross between *L. esculentum* and an introgression line that contains the long arm of chromosome 11 (line 11, Eshed et al., 1992). Analysis of the F2 population corroborated the division of *SL8* markers into clusters. *SL8C* and *SL8D* cosegregated completely with the RFLP markers *TG546* and *6-16*, respectively, and *SL8E* mapped between markers *TG26* and *TG105*, 0.25 cM from *TG26* (Figure 3). The susceptible-type *L. esculentum* is a common parent between the IL population and the F2 population. A comparison of the *SL8* RFLP patterns of the ILs (Figure 3) with those of resistant and susceptible *L. esculentum* plants (Figure 2), shows clearly that nearly all fragments that belong to group *SL8D* are polymorphic between the resistant and susceptible F2 parents (compare Figure 2 and Figure 3). The nonpolymorphic bands in Figure 2 belong mostly to the other groups. This indicates that the region containing cluster *SL8D* is the region which was originally introgressed from *L. pimpinellifolium* into *L. esculentum*.

#### **Example 4. Heterogeneity in recombination rates in the *I2* region**

The locus *SL8-D*, containing at least 4 clustered members of the *I2C* family, spans a 0.03 cM region in the *I2* locus. Two cosmid clones of approximately 20 kb insert from this cluster, *I2C-134* and *I2C-150* (Fig. 11), contain only one copy of *SL8*. Hence, 0.03 cM genetic interval spans at least 20 kb, which assigns an estimation of at least 670 kb/cM in the region containing this group. This ratio is similar to the average of 550 kb/cM over the entire genome. In contrast, in proximal regions the ratio is <150 kb/cM between *D2* and *SL8* (*I2C-1*; Figure 1) and approximately 43 kb/cM between *TG105A*

and *TG26* (Segal et al., 1992). High variability in the physical to genetic ratio is common in the context of different regions of the chromosome, and is shown here to fluctuate regionally as well.

5    **Example 5. *I2C* Genes share structural similarity with a family of plant resistance genes**

Candidate members of the *I2C* gene family were isolated from genomic libraries of *F.o.I* race 2 resistant tomatoes utilizing SL8 as a probe. The isolated clones were compared to the genomic DNA on southern blots, and clones that contain resistant-type polymorphic bands from the *SL8D* locus were further characterized. The cosmid clone  
10    *I2C*-134 (Figs. 11, 12) contains the *I2C-1* gene, that includes in its 3' region the SL8 marker (Fig. 1C). It was found to contain the polymorphic bands TR1 and TR5 (Fig. 2). *I2C*-134 also exhibits resistant-type polymorphic bands after digestion with other endonucleases, such as HindIII, DraI and EcoRI (data not shown). Cosmid *I2C*-150 (Fig.  
15    11) contains another gene, *I2C-2*, represented by the polymorphic bands TR4 and TR7 (Fig. 2). As the recombination in individual BR 30(5) has occurred between band TR7 and the other resistant-type bands, it would be expected that the gene which contains TR7 will not contain other resistant-type bands. The presence of both TR4 and TR7 bands in *I2C*-150 could be explained either by a recombination within the gene *I2C-2* in the  
20    individual BR 30(5), or by comigration of bands of different origin. Other cosmids isolated contained non-polymorphic bands, and were not sequenced.

One continuous open reading frame was identified in each of the genes *I2C-1* and *I2C-2*. Fig. 8 shows a comparison between the deduced amino acid sequences of the *I2C-1* and the *I2C-2* genes and recently isolated plant resistance genes. Although the overall  
25    homology is rather low, an intriguing structural similarity is apparent. All genes contain in their N terminus a conserved nucleotide binding site, P-loop, and other conserved amino acid stretches of unknown function, which are shown in Fig. 8 as boxes I-VI. In their C terminus they all display a long region of leucine-rich-repeats (LRR), which spans at least half of the gene. The LRR of *I2C-1* are aligned in Fig. 9. The N terminal  
30    parts of the *I2C* repeat segment are comparable to the consensus LRR found in the resistance genes *RPS2*, *N* gene and *L6*, and to the consensus of the *T-LR* VSG expression site associated gene from *Trypanosoma* (Fig. 9, Ross et al., 1991; Smiley et al., 1990).

The latter protein shares 52 % similarity and 25 % identity with the 3' part of *I2C-1*. The C-terminal parts of the repeat segments are not conserved, and are of variable length.

**Example 6. Transcribed sequences from the I2C gene family contain insertion and frame-shifts.**

In order to compare different resistant-type members of the I2C family, three different cDNA libraries of resistant tomato varieties were screened with the SL8 probe. Fifteen independent clones were isolated, and all of them were shorter than *I2C-1*, containing only the 3' ends of the genes. The reason for obtaining only partial clones is not known, as much longer inserts have been isolated from these libraries. Two of the longest clones, designated *I2C-3* and *I2C-4*, of 1200 and 1600 bp long, respectively, were sequenced. Interestingly, one of the cDNA clones, *I2C-4*, contains two frame shifts, and thus, if translated, would result in a truncated peptide. In Fig. 10, *I2C-4* is artificially shown as a continuous chimera of the 3 different ORFs, and the junctions are indicated with arrows. The comparison of the 3' region of the two genomic and two cDNA clones is shown in Fig. 10. Striking insertions or deletions, chiefly in the C-terminal region, can be observed. Most insertions are shared by at least two different genes, though the combinations are different for each insertion. Interestingly, close to the C-terminus the different genes contain between 2 and 6 repeats of an almost identical sequence. These repeats are indicated in Fig. 10. The largest insertions in genes *I2C-3* and *I2C-4* are made up exclusively of these repeats.

**Example 7. Plant transformation procedures**

In order to correlate the disease resistance capacity of the I2C genes, they have been transformed into tomato plants in a few different formats:

(a) Cosmid clones I2C-134 and I2C-150 containing the complete inserts of clones I2C-1 and I2C-2, respectively, in the BamHI site of cosmid TDNA 04541 (Jones et al., 1992) (Fig. 11), were directly transformed into sensitive tomato lines VF-36 and Money Maker (Jones et al., 1992). Another 10 cosmids have been isolated by homology to I2C and were similarly transformed. To this end the cosmids were transferred into *Agrobacterium tumefaciens* using standard transformation procedures. The binary vector

A. tumefaciens LBA 4404 is suitable for the transformation procedure. Tomato explants are inoculated as described in Jones et al., 1992.

(b) Similarly, clone I2C-1 was introduced into the PGA492 binary vector supplemented with the B-domain of the 35S promoter (constructs 134 A and 134H) and were directly transformed into the same sensitive tomato lines as described above.

Fig. 12 depicts sense constructs from the I2C-134 cosmid containing the I2C-1 clone in the PGA492 binary vector. A depicts an *AccI* subclone, 134A, containing approximately 3kb upstream and 300 bp downstream to the open reading frame (ORF), cloned into the *Clal* site of the PGA492 vector; B. depicts a *HincII* subclone, 134H, containing around 3kb upstream and 800 bp downstream to the translated region, cloned into the *HpaI* site of the PGA492 vector; D depicts a small subclone from the BS subclone of cosmid 134 depicted in C, containing 300 bp upstream and 800 bp downstream to the translated region, cloned downstream from the B domain (B Dom) of the 35S promoter, in the PGA492 vector, to create 134B.

(c) Antisense and sense partial clones were constructed with partial sequences from I2C-1, I2C-2, and I2C-3, and subcloned into 35S omega expression vectors in the PGA492 binary vector. These constructs (2-1, 6-3, 5-1 and 31-17) were transformed into resistant tomato lines (Motelle) as described in section (a) above.

Fig. 13 depicts the antisense and sense constructs 2-1, 6-3, 5-1 and 31-17 for transformation of Fusarium resistant tomatoes. On the top is a map of SL8-134 (I2); indicated is the *HindIII* fragment used for the antisense and sense cloning in 2-1 and 6-3, which spans nucleotides 2540-3716 in the I2C-1 sequence shown in Fig. 4. Constructs 5-1 and 31-17 contain the full-length insert of cDNA I2C-3. 35S is the cauliflower mosaic virus (CaMV) 35S promoter,  $\Omega$  is a translation enhancer from tobacco mosaic virus, and nos 3' is a terminator, 3' untranslated sequence of the nopaline synthase gene. All the clones were prepared in the PAG492 binary vector using the unique *XbaI* site.

The resulting transformed tomato lines are tested for complementation of Fusarium resistance in sensitive lines like Money Maker or abrogation of Fusarium resistance in the Motelle line. Tests are carried out by inoculating 10 days old seedlings with freshly prepared Fusarium fungus cultures and disease is estimated during 10-20 days following inoculation. Sensitive plants show retarded growth browning of vascular tissue and usually dye within 20 days.

## REFERENCES

- An, G., (1986) *Plant Physiol.* 81, 86-91.
- Behare, J., Laterrot, H., Sarfatti, M., and Zamir, D. (1991). Restriction fragment  
5 length polymorphism mapping of the *Stemphylium* resistance gene in tomato. *Mol. Plant-Microbe Interact.* 4, 489-492.
- Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J.,  
Leung, J., and Staskawicz, B.J. (1994). RPS2 of *Arabidopsis thaliana*: A leucine-rich  
repeat class of plant disease resistance genes. *Science* 265, 1856-1860.
- 10 Bournival, B.L., Vallejos, C.E., and Scott, J.W. (1990). Genetic analysis of resistances  
to races 1 and 2 of *Fusarium oxysporum* f. sp. *lycopersici* from the wild tomato  
*Lycopersicon pennellii*. *Theor. Appl. Genet.* 79, 641-645.
- Briggs, S.P., and Johal, G.S. (1994). Genetic patterns of plant host-parasite interactions.  
*TIBS* 10, 12-16.
- 15 Busch, S.J., and Sassone-Corsi, P. (1990). Dimers, leucine zippers and DNA-binding  
domains. *TIG* 6, 36-40.
- Colicelli, J., Field, J., Ballester, R., Chester, N., Young, D., and Wigler, M. (1990).  
Mutational mapping of RAS-responsive domains of the *Saccharomyces cerevisiae*  
adenylyl cyclase. *Mol. Cell. Biol.* 10, 2539-2543.
- 20 Dangl, J.L. (1995). Piece de Resistance: Novel classes of plant disease resistance genes.  
*Cell* 80, 363-366.
- Dekeyser, R.A. et al., (1990) *Plant Cell* 2, 591-602.
- Depicker, A., Stachel, S., Dhaese, P., Zambryski, P. and Goodman, H.M. (1982)  
Nopaline synthase: Transcript Mapping and DNA Sequence. *J. Molec. Appl. Genetics*  
25 561-573.
- Devereux, J., Haeberli, P. and Smithies O. (1984). A comprehensive set of sequence  
analysis programs for the VAX. *Nucl. Acids Res.* 12, 387-395 .
- Elias, K.S., Zamir, D., Lichtman-Pleban, T., and Katan, T. (1993). Population  
structure of *Fusarium oxysporum* f. sp. *lycopersici*: restriction fragment length  
30 polymorphisms provide genetic evidence that vegetative compatibility group is an  
indicator of evolutionary origin. *Mol. Plant-Microbe Interact.* 6, 565-572.



- Ellis, J.G., Lawrence, G.J., Finnegan, E.J., and Anderson, P.A. (1995). Contrasting complexity of two rust resistance loci in flax. *Proc. Natl. Acad. Sci. USA* 92, 4185-4188.
- Eshed, Y., Abu-Abied, M., Saranga, Y., and Zamir, D. (1992). *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. *Theor. Appl. Gen.* 83, 1027-1034.
- Eshed, Y., and Zamir, D. (1994). A genomic library of *lycopersicon pennellii* in *L. esculentum*: a tool for fine mapping of genes. *Euphytica* 79, 175-179.
- Eshed Y. and Zamir, D. (1995). An introgression line population of *L. pinnellii* in the cultivated tomato enables the identification and fine mapping of yield associated QTL. *Genetics*, in press.
- Eyal, Y., Meller, Y., Lev-Yadun, S. and Fluhr, R. (1993) A basic-type promoter directs ethylene responsiveness and abscission zone-specific expression. *The Plant J.* 4,225-235.
- Gallie, D.R. et al. (1987) *Nucl. Acid Res.* 15, 3257-3273.
- Gordon-Kahn, W.J. et al. (1990) *Plant Cell* 2, 603-618.
- Grant, M.R., Laurence, G., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R.w., Dangl, J. (1995). Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. *Science* 269, 843-846.
- Greve, H.D. et al. (1983) *J. Mol. Appl. Genet.* 1, 499-511.
- Hayashimoto, A. et al. (1990) *Plant Physiol.* 93, 857-863.
- Horsch, R.B. et al., (1985) *Science* 227, 1229-1231.
- Johal, G.S., and Briggs, S.P. (1992). Reductase activity encoded by the *HMI* disease resistance gene in maize. *Science* 258, 985-987.
- Jones, D.A., Thomas, C.M., Hammond-Kosack, K.E., Balint-Kurti, P.J., and Jones, J.D.G. (1994). Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* 266, 789-793.
- Jones, J.D.G., Shlumukov, L., Carland, F., English, J., Scofield, S.R., Bishop, G., and Harrison, K. (1992). Effective vectors for transformation, expression of heterologous genes, and assaying transposon excision in transgenic plants. *Transgenic Research* 1, 283-297.
- Kobe, B., and Deisenhofer, J. (1993). Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* 366, 751-756.

- Kobe, B., and Deisenhofer, J. (1994). The leucine-rich repeat: a versatile binding motif. *TIBS* 19, 415-421.
- Kobe, B., and Deisenhofer, J. (1995). A structural basis of the interactions between leucine-rich repeats and protein ligands. *Nature* 374, 183-185.
- 5 Laterrot, H., and Moretti, A. (1995). Confirmation of the linkage *Tm-2, ah, Fr1*. *TGC* 45, 29.
- Lawrence, G. J., Finnegan, E. J., Ayliffe, M. A., and Ellis J. G. (1995). The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. *Plant Cell* 7, 1195-1206.
- 10 Martin, G. B., Ganai, M. W. and Tanksley, S. (1992). Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci. *Mol. Gen. Genet.* 233, 25-32.
- Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Frary, A., Ganai, M.W., Spivey, R., Wu, T., Earle, E.D., and Tanksley, S.D. (1993). Map-based cloning of a
- 15 protein kinase gene conferring disease resistance in tomato. *Science* 262, 1432-1436.
- Mindrinos, M., Katagiri, F., Yu, G.-L., and Ausubel, F.M. (1994). The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine rich repeats. *Cell* 78, 1089-1099.
- Ori, N., Paran, I., Aviv, D., Eshed, Y., Tanksley, S., Zamir, D., and Fluhr, R. (1994).
- 20 A genomic search for the gene conferring resistance to *Fusarium* wilt in tomato. *Euphytica* 79, 201-204.
- Ross, D.T., Raibaud, A., Florent, I.C., Sather, S., Gross, M.K., Storm, D.R., and Eisen, H. (1991). The trypanosome VSG expression site encodes adenylate cyclase and a leucine-rich putative regulatory gene. *EMBO J.* 10, 2047-2053.
- 25 Saraste, M., Sibbald, P.R., and Wittinghofer, A. (1990). The P-loop a common motif in ATP- and GTP-binding proteins. *TIBS* 15, 430-434.
- Scott, J.W., and Jones, J.P. (1991). Genetic control of resistance to races 1, 2 and 3 of *Fusarium* wilt. *TGC* 41, 47.
- Segal, G., Sarfatti, M., Schaffer, M.A., Ori, N., Zamir, D., and Fluhr, R. (1992).
- 30 Correlation of genetic and physical structure in the region surrounding the *I<sub>2</sub>* *Fusarium oxysporum* resistance locus in tomato. *Mol. Gen. Genet.* 231, 179-185.

- Smiley, B.L., Stadnyk, A.W., Myler, P.J., and Stuart, K. (1990). The trypanosome leucine repeat gene in the variant surface glycoprotein expression site encodes a putative metal-binding domain and a region resembling protein-binding domains of yeast, *Drosophila* and mammalian proteins. *Mol. Cell. Biol.* **10**, 6436-6444.
- 5 Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G., and Jones, J.D.G. (1995). Molecular Genetics of Plant Disease Resistance. *Science* **268**, 661-666.
- Tanksley, S.D., Ganai, M.W., Prince, J.C., de Vicente, M.C., Bonierabale, M.W., Broun, P., Fulton, T.M., Giovanonni, J.J., Grandillo, S., Martin, G.B., Messeguer, R., Miller, J.C., Miller, L., Paterson, A.H., Pineda, O., Roder, M.S., Wing, R.A.,
- 10 Wu, W. and Young, N.D. (1992). High density molecular linkage maps of the tomato and potato genomes. *Genetics* **132**: 1141-1160.
- Tanksley, S.D., Ganai, M.W. and Martin, G.B. (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *TIG* **11**, 63-68.
- Whitham, S., Dinesh-Kumar, S.P., Choi, D., Hehl, R., Corr, C., and Baker, B.
- 15 (1994). The product of the tobacco mosaic virus resistance gene *N*: Similarity to toll and the interleukin-1 receptor. *Cell* **78**, 1101-1115.
- Young, N.D., Zamir, D., Ganai, M.W., and Tanksley, S.D. (1988). Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. *Genetics* **120**, 579-585.

## CLAIMS:

1. A DNA molecule selected from the group comprising:
  - (i) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-1 (SEQ. ID. NO.:1);
  - 5 (ii) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-2 (SEQ. ID. NO.:2);
  - (iii) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-3 (SEQ. ID. NO.:3);
  - (iv) a DNA molecule having a nucleotide sequence derived from the coding region of the clone herein designated I2C-4 (SEQ. ID. NO.:4);
  - 10 (v) a DNA molecule characterized by containing a coding sequence representing at least 60% similarity with the encoded open reading frame in the DNA sequence of at least one of the DNA molecules (i) and (ii);
  - (vi) a DNA molecule capable of hybridization with any one of the DNA molecules (i)- (v) under moderately stringent conditions;
  - 15 (vii) a DNA molecule that differs, by insertion, deletion or as a result of the degenerative nature of the genetic code, from the DNA sequences (i)-(vi); and
  - (viii) a fragment of any of the DNA molecules (i)- (vii).
- 20 2. A DNA molecule according to claim 1 which is expressed in tomato plants and confers resistance to *Fusarium oxysporum* f.sp *lycopersici* race 2 in said tomato plants.
3. A gene construct containing a DNA molecule according to claim 2.
- 25 4. A gene construct according to claim 3 wherein said DNA molecule includes regulatory sequences that flank the coding region.
5. A cosmid into which is subcloned a gene construct according to claim 4.
- 30 6. A gene construct according to claim 3 which further comprises DNA regulatory sequences enabling the expression of said DNA molecule in plant cells.

7. An expression vector comprising a gene construct according to claim 6 wherein said DNA regulatory sequences comprise a plant promoter, a DNA sequence that enhances translation of the mRNA transcribed from said DNA molecule and a polyadenylation/terminator sequence.

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8. A tomato cell line or plant line transformed with a cosmid according to claim 5 or with an expression vector according to claim 6 or 7.

9. Tomato plants regenerated from transformed cells according to claim 8.

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10. A method of selective breeding of Fusarium resistant tomatoes employing a DNA molecule according to claim 1 as a direct restriction fragment length polymorphism (RFLP) probe, which comprises:

(i) marking said DNA molecule with a suitable marker; and

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(ii) reacting said probe of (i) with DNA extract of a tomato plant under hybridization conditions;

thus obtaining restriction-length polymorphism that is indicative of a resistance-type gene, which facilitates the selection of progeny that contains said resistance gene.

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11. A method of screening new diseases in plants of the Solanaceae family employing a DNA molecule according to claim 1 as a direct RFLP probe which comprises:

(i) marking said DNA molecule with a suitable marker; and

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(ii) reacting said probe of (i) with DNA extracts from said Solanaceae species,

thus identifying the homologous gene family in those species which can be linked to known resistance genes in those species.

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12. Use of a DNA molecule according to claim 1 as a direct RFLP probe for selective breeding of Fusarium resistant tomatoes.

13. Use of a DNA molecule according to claim 1 as a direct RFLP probe for screening new diseases in plants of the Solanaceae family.

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FIG. 1A

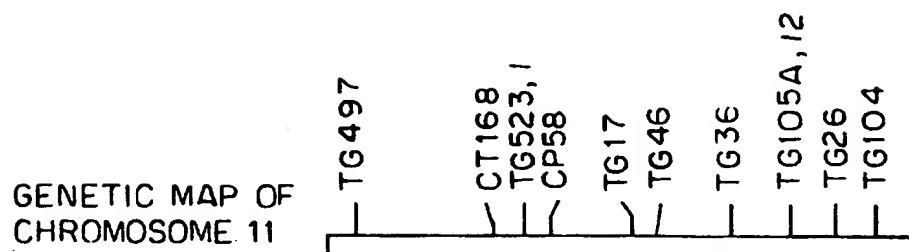


FIG. 1B

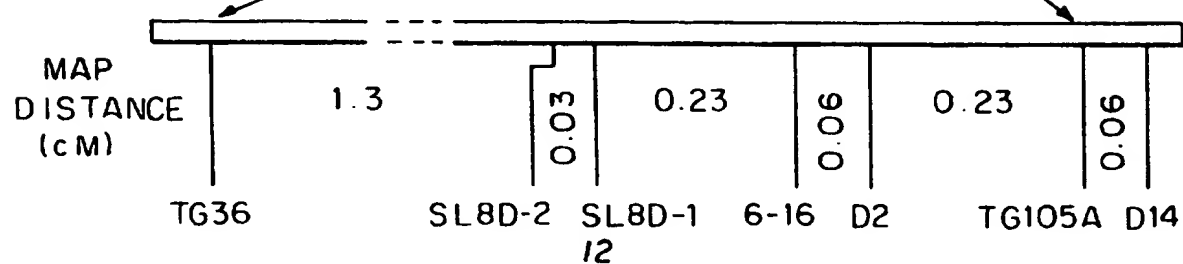


FIG. 1C

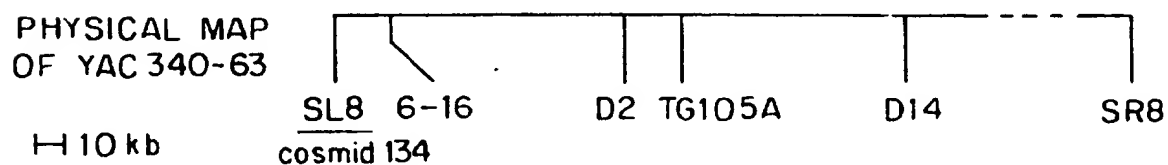


FIG. 2

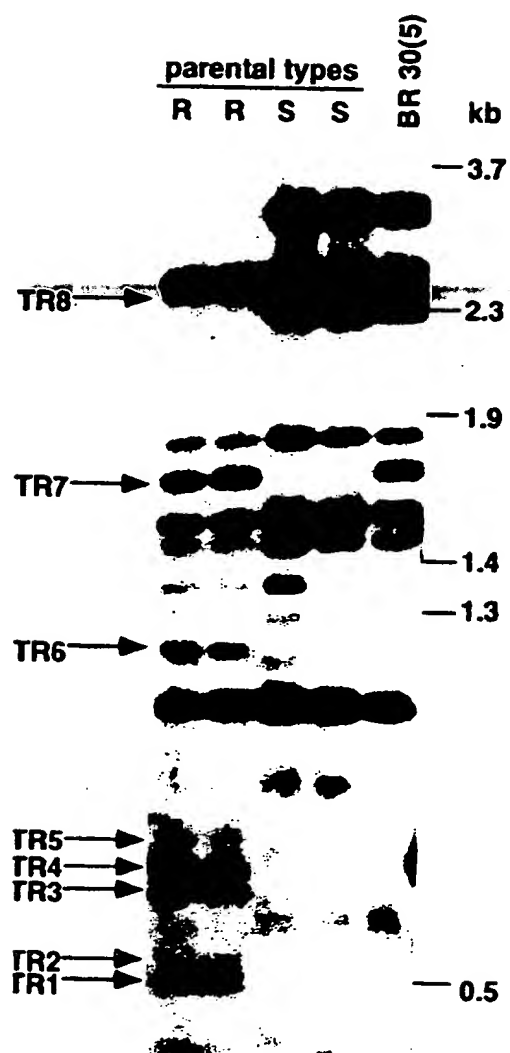




FIG. 3A

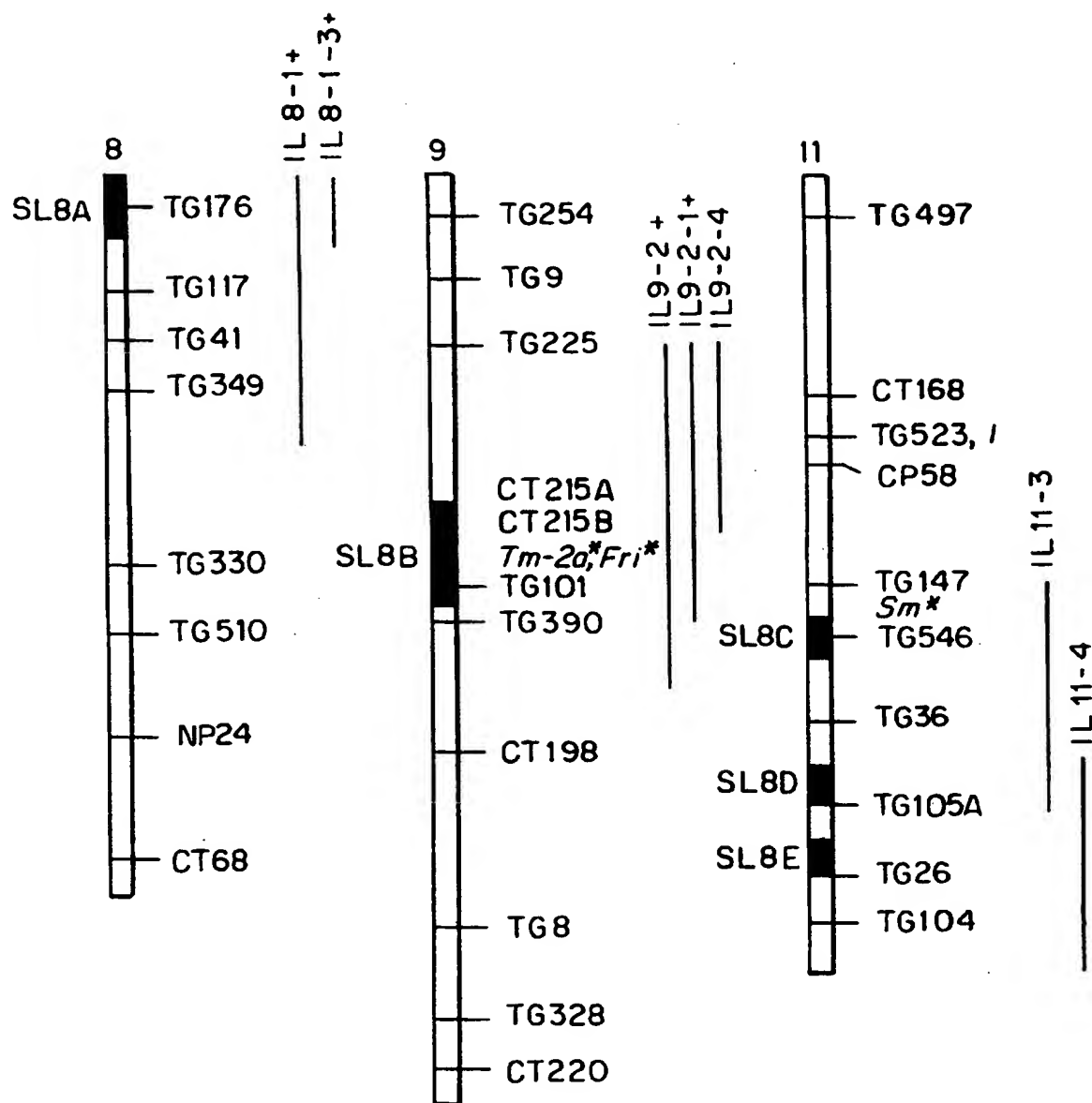


FIG. 3B

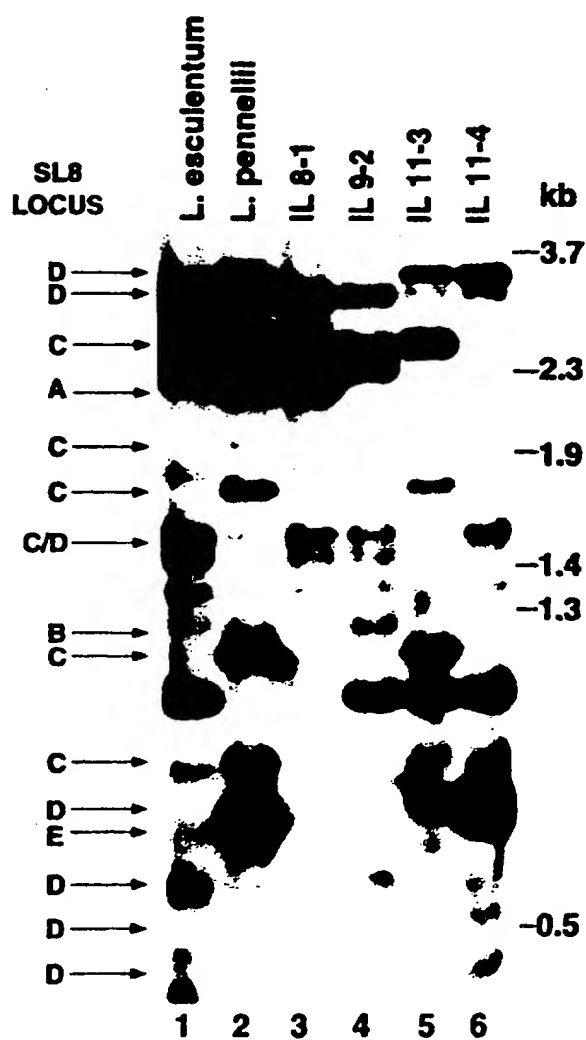


FIG. 4A

[illegible]

FIG. 4B

1682 CAATCGATATCCAGTTCATTAACAAAAAATAAGCAAGAGGGTGTTCATACATACTGCCTACACAAAGATCCTTGAGGGCACTATCATTTGTCTCATTAACGATT  
1792 I D I O F H Y S K K L S K R V L H N I L P T M R S L R A L S L S H Y Q I  
GAGGTGTGCCAAATGACTGTTATCAAAATAAGCTCCTCAGATTTTGGACCTTCTGAGACATGATATACAAAGTTGCCGATTCCTATTTTGTGTGTATTAACCTT  
11902 E V L P N D L P I K L K L L R F L D L S E T S T K L..R..D..S..I..F..V..L..Y..N..L  
AGAGACACTTCTCTGTCATCTTGTAATATCTTGAGGAGCTACCGCTGCAGATGGAGAACTGATTACTTGGCTCATCTTGACATAAGCAACACTCGGCGCTTGAAGA  
.E..T..L..L..S..S..C..E..Y..L E E L P L O M E K L I N L R H L L D I S N T R R L K I  
TCCACTACATCTGAGCAGGTGNAAGAGCTCCAAAGTGTGGTGAGCCAAAGTTCTTGTAGGTGGTGGAGAAATGGAAATATTTGGTGAAGCACCACCACTTATATGGA  
2012 P L H L S R L K S L Q V L V G A K F L V G G W R M E Y L G E A P N L Y G  
TCTCTATCAATCTAGAGTTGGAAAATGTGTTGATAGAAGGGAGCTGTGAAGGCAAGATGAGGGAGAAAGTATGTTGAGCAATATATCATTTGGAGTGGAGTGAAG  
2122 S L S I L E L E N V V D R R E A V K A K M R E K N H V E Q L S L E W S E S  
CATTAGTCTGACAAATTCACAAAGAACATCTTGATGAGCTACGCCACATAAACATTAAGCACTTGAATCACTGGATATAGAGGGACAAACTTTCCAA  
2232 I S A D N S O T E R D I L D E L R P H K N I K A V E I T G Y R G T N F P P N  
ACTGGGTAGCTGATCCTTTTGTAAAGCTGGTGCATTTGTATCTTAGAACTGCAAGGACTGTTAGTCTCTCCAGCACTAGGACAACTCCCTTGTGTGAATTCCTT  
2342 W V A D P L F V K L V H L Y L R N C K D C Y S L P A L G Q L P C L E F L L  
TCCATTAGAGGATGCATGGATAAGAGTGGTGACAAAGAGTCTATGGCAGATTGTCTCCAAAAGCCCTTTAACTCTCTTGTGAAGCTAGATTTGAAGATATGCC  
2452 S I R G M H G I R V V T E E E Y G R L S S K K P F N S L V K L R F E D M P  
TGAATGAAGCAATGGCACACACTAGGAATTTGGAGAGTTCCTACACTTGAGAAACTTTCCATTAAATTTGCCCTGAGCTCAGTTTGGAGATACCCTTCCAAATTTCAA  
2562 E W K O W H T L G I G E F O T L E K L S I K N C P E L S L E I P I Q F S S  
GTTTAAAAGGTTAGATATATGTGATTGTAAGTCTGTACCTCTTCTCTTTAGCATACTGCCCACTACCTTCTATL K R I K I S G C P K L S  
2672 L K R L D I C D C K S V T S F O F S I L P T T L L K R I K I S G C P K L S  
TTGAGGCCAGTGTGGAGATTTTGTGGAGTATTTGAGTGTGATTTGTTGTGTGATGATATATCATCTGAGTTTCTCCCAACAGCAGCTCAATTTGATAT  
2892 L E A P V G E M F V E Y L S V I D C G C V D D I S P E F L P T A R Q L S I  
TGAGAAATGCCCAACGTTTACFAGGTTTTGATTCTCTGCCACTGCAAGTCTCCATATTCGAAATTTGAAATCTCGATGCGCATGTGGAGGAGCGGCCAGCTGA  
3002 E N C H N V T R F L I P T A T E S L H I R N E K L S M A C G G A A Q L T I  
CGTCACTGAATATTGGGGATTAAGAGCTCAAGTGTCTCCAGAACTCTTCCATCTCTCAAGAACTCGGACTGACTTATTTGTCCAGNAATAGAGGAGAAATGCCCC  
3112 S L N I W G C K K L K C L P E L L P S L K E W R L T Y C P E I E G E L P  
TTCAATTACAAATCTCGATATCAGATATTGCAAGAACTGTGAATGGCGAAAGGAGTGGCATTTACAGAGACTCACAGAGTTATGGATCAACATGATGGAGTGA  
3222 F N L Q I L D I R Y C K K L V N G R K E W H L Q R L T E L W I K H D G S D  
CGAACATTTGAACATTTGGAGTTCCTCTCTATTACAGAGACTATTTCATATTCAATCTGAAACATTAAGCAGCCAACTCAAAAGCCCTCACCCTCTCTCAATTC  
3332 E H I E H W E L P S S I O R L F I F N L K T E S S O H L K S L T S L O F L  
TACGATTTGTGGTAATTTATCTCAGTTTCAGTCACAAAGGCCAACTTCTCTCTCTCAGCTTCACTGCTTCAAACTCTACAAATCTGGAATTTCTTAACTTCAA  
R I V G N L S O F O S O G O L S S F S H L T S L O T L Q I W E L N L Q

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3442 TCACCTACCTGAATCAGCAGCTGCCCTCCTCCCTCTCTCCTGATCATCTCCAAATGCGCTTAATCTCCATTAAGGGATGCGCTCTCCCTCTCTACACT  
S L P E S A L P S S L E H L I I S N C P N L Q S L P L K G M P S S L S T L  
3552 ATCAATTTCCAAATGTCATGCTCAGCAGCTACTAGAAATTTGACAGGGGGAATCTGACAGAAATTTGCTCATATCCCACTATCCCACTACAGATCGATGAGGAATGCAATG  
S I S K C P L L T P L L E F D K G E Y W T E I A H I P T I Q I D E E S M  
3662 AATGATTAAACAATGGCTCCCACTGATGTAGCTATTCTTTCCCTCATAGCTTTTATTTCACCTTTGCTTTGGGTTACTTCTCTTTTCATTTTAAATCATGCGG  
3772 GGCTAGCTCATCTCAACACATAGCATATATTAACCTCCATAGAGAAATCTAAATTTTAAAGGATAATTCATACACAGTTTATAGGAATAACTGCAACTTCGCAAT  
3882 GTCACATGTTATATGATCTATGTTCTCATGGCTTATTGGTTTATGCTCTTACCGTGTGTAATTCACCGTCTCAATGCGCACCATGTTTAAATCAAAAGTTTTTATGTTCT  
3992 TGTAAATCATCAACCATCTCTATGTCTAGCAATTTGGATAGATAAAGAGGTAGACAAAAGAGGAAACATCTTTTCTGTTATAGATATCTCTCTGCTATATCTTTGGCAATTTTAAAGCTACA  
4102 GATAGGTAAGGGCTATAGATATACATTTGCGAGGTGTTAATCCAAAGGTAGAAATCCCTGTCTTTAGATATCTCTCTGCTATATCTTTGGCAATTTTAAAGCTACA  
4212 GTTTGAACCTCATGTGTTGCTTAACTTAAACATGTTTGTGCTTAAATCAGATGTGGATTTGAAGAGCGAGTACGACAACTCTGGTACATTAATTTGTCCTAGGAACTG  
4322 TTTCTAAGGTGCTGCTGCTATTTTACATCTGTTCCCGAGTTTGGTTTATTTTAAATCTTTCCACTAAAGCTATTAATGTCCTCCACAGTGAATTTTTCAGGTCCTGTTGT  
4432 TATAGGCAAGTCTTTGAGATGCGACTATCAAGAGAGGGCGATTACAAATCAGTGTACCGCTGAACATAATTCATGTTCCAGTGCAGGCTCTTTTGTAGTTGACAAACT  
4542 CGATTAGTTAATATGTTTGGGACTCACTAGTGGTTAGAGTACTCATTTTGTAGAGCTTGTGTACAGAAAATCAAAATTAGAAATTTATCACTCGCGATGGTTGGAAATTAAC

FIG. 4C

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## FIG. 5A

-202 AAGAGGGAAGTTTGGCTTCTGTAGATTAGTAAACATAGTGTGATTAACAGAGAGCAATGGCTGATCATCTCTGTTTGCMAATTACTCTTAGCATTCGACAG  
 -92 GTAAATAAAGCTCATACAGACACTATTGAAATATATTTGTTAAAGAAATCATATTGCTGTTTCCCTGTTGTTGCTTTTCAGATTTGAGAAATGGAGATTGGCTTAGC  
 -30 M E I G L A  
 18 APTTGGTGGTCATTCTCTCTCAGCTTTGAAATGTTCTGTTTGATAGGCTTGCTCTAACGGTGTATCTGCTCAACATGTTTGGNAGCATTAAGGATCATGTTTAAGCTCT  
 7 V G G A F L S S A L N V L F D R L A P N G D L L N M F R K H K D H V K L L  
 128 TAAAGAGCTGAATAAGCTTGGCTGATTAAGTATGATGAGCAAGCATCAATTCATCTGTGAGAGACTGGCTTAATAGCTTCCAGAT  
 44 K K L K H T L R G I Q I V L S D A E N K Q A S N P S V R D W L N E L R D  
 238 GCTGTGACTCTGCTGAATAATTATAGAGAAGTCAATTATGAAGCTTTAGGCTTAAAGTGAAGGTGAGCATCAGAACTTTTCAGAAACAGCAACCCAGCAAGTAA  
 80 A V D S A E N L I E E V N Y E A L R L K V E G Q H Q N F S E T S N Q Q V S  
 348 TGATGAATTTTCTTAACATAAAGGACAGTGAAGACACTATTGAACATTAAGGATTTCCAGAGCAATTTGGTCTGCTTAAAGGAGTATTTGATTCCA  
 117 D E F P L N I K D K L E D T I E T L K D L Q E Q I G L L G L K E Y F D S T  
 458 CGAACTAGAAACTAGAACACCTTCAACTTTGATGATGAACCATATCTTTGGTAGGACGAGCGAAATAGAGGATTTGATTTGACCGCTATTGCTGGAAGGTGCA  
 154 K L E T R T P S T S L I D E P D I F G R Q S E I E D L I D R L L S E G A  
 568 AGTGGAAATCTGACAGTGGTCTTATTTGGAATGGGTGGCTTGGCAAGACAACTGCTAAAGCCGTATACAAATGATGAGAGTGTGAAGAACCAATTTTGATT  
 190 S G K N L T V V P I V G H G L G K T L A K A V Y N D E S V K N H F D L  
 678 GAAAGCTTGGTTTGGTTCGAGGCGTATAATGCTTTTCAGAAATCAAAAGGGTTACTTCAAGAAATTTGGCTCAATTGACTTAGTTGATGACAAATCTTAATCAGCTAC  
 227 K A W F C V S E A Y N A F R I T K G L L Q E I G S I D L V D N L N Q L Q  
 788 AAGTCAATTAAGGAAGATTAAGGAAGAAAGTTCCTTATGCTTCTGATGATGTGGAATGACAACTACAAAGTGGGATGAAATGAGAAATGTTTTTGTACAA  
 264 V K L K E R L K E K F L I V L D D V W N D N Y N E W D E L R C N V F V Q  
 898 GGAGATATAGGAATGATGATGACAGACGCAAGACAGTGTGCTGATGATGGGAAATGAGCAAAATAGCAATGGCAATTTGTCACGAGCTCTTGGTC  
 300 G D I G S K I I V T T R K D S V A L M H G N E Q I S H G N L S T E A S W S  
 1008 TTATTTCAAGACATGCAATTTGAACATGATGCTTATGGACATTCGGAATTTGAAGGTTGGAGCAAAATTCGAGTAAAGTGCNAGGACTGCCCTAGCTCTGA  
 337 L F Q R H A F E N M D P M G H S E L E E V G R Q I A A K C K G L P L A L K  
 1118 AGAGCTTGGTGGCATGTTACGCTCCAAATCAGAGGTTGAGAGTGGAAATGATTTCTGAGAAAGTGAATATGGAGCTGCGAGACAAATGACATATTACCCAGCGTTAATG  
 374 T L A G H L R S K S E V E E H K C I L R S E I W E L R D N D I L P A L M  
 1228 TTGAGCTACAATGATCTTCTGTCACATTTAAAGCGATGCTTTCTTTTGTGCAATTTTCTTAAGATTTATCCATTTAGGAAGAAACAAAGTATTATTCATCTATGATTCG  
 410 L S Y N D L P A H L K R C F S F C A I F P K D Y P F R K E Q V I H L W I A  
 1338 CAATGGTCTGTACCTGGAAGATGAATAATTCAAGATTAGGCAACCAATCTTTCTGAGTTGAGTTCAAGATCAATTTTGAAGGTTCCCAATCTCTCTGAG  
 447 N G L V P V E D E I I Q D L G N Q F F L E L S S R S L F E R V P N P S E G  
 1448 GAAACATAAGGAATTTATCTTAATGATGACCTTGTCAATGATTTAGCCCACTTGCATCTTCNAACTTTGTATCAGTTGGAAGAGAGCCAGGATCTCATATGTTG  
 484 N I K E L F L M H D L V N D L A Q L A S S K L C I R L E E S Q G S H M L  
 1558 GAACAAATGCGCACTTATCATATTCTATGGGATATGACGGTGGTGTGAGAAATGACACCCCTCTACAAATTTGGAGCACTGAGGACATTTGCTTCCGACATGTAGTAG  
 520 E Q C R H L S Y S M G Y D G G F E K L T P L Y K L E Q L R T L L P T C S S  
 1668 TGTCATTTATTAACCTCTAACAGAGGTTGTCATACACTGCTTACACTAGATCTTTAAGGCGCTTATCATTTGCTTATCAAGATGAGAGGAGTTGC  
 557 V N Y F Y N P L T K R V L H N I L P T L R S L R A L S L S H Y K M E L P  
 1778 CAATGACTTGTTCATTAATTAAGCTCTCAGATTTTGGATTTTTCGAGCAAAATTAAGGTTGCCAGATTTCCATTTGTGTGTATTAATCTGAGAGACACTT  
 594 N D L F I K L K L R F L D I S R T N I K R L P D S I C V L Y N L E T L  
 1888 CTCCTTTCATCTTGTAACTTGAGGAGCTACCGCTGACATGAGAGTGAATTAACCTTGGCTCATCTTGACATTAAGCAACACTTGGCACTTGAAGATGCCACTACATCT  
 630 L L S S C K L E E L P L Q M E K L I N L R H L D I S N T W H L K M P L H L



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## FIG. 6

1	AATTCGGCAC	GAGAATTGAA	ATTGGAGGCT	CCAGTTGGTG	AGATGTTTGT
51	GGAGTATTTG	AGTGTGAATG	ATTGTGGTTG	TGTAGAAGAT	ATATCACCTG
101	AGTTTCTCCC	AACAGCACGT	AAATTGATTA	TTACGGATTG	CCAGAACGTT
151	ACTAGGATTT	TGATTCCCTAC	TGCCACTGAA	ACTCTCACTA	TTGAGAATTG
201	TGAGAATGTT	GAAAACTAT	CGGTGGCATG	TGGAGGAGCG	GCCCAGATGA
251	CGTCTCTGAT	TATTTCCGAG	TGTAAGAAGC	TCAAGTGTCT	TCCAGAACGT
301	ATGCAGGAAC	TCCTTCCATC	TCTCAAGGAA	CTGCGTCTGT	CTGATTGTCC
351	AGAAATAGAA	GGAGAATTGC	CCTTCAATTT	ACAAAACTC	TATATCAGTT
401	ATTGCAAGAA	ATTGGTGAAT	GGCCGAAAGG	AGTGGCATT	ACAGAGACTC
451	ACAGAGTTAT	GGATCCATCA	TGATGGGAGT	GACGAAGATA	TTGAACATTG
501	GGAGTTGCCCT	TCCTCTATT	AGAGTCTTAC	CATATGCAAT	CTGATAACAT
551	TAAGCAGCCA	ACATCTCAAA	AGCCTCACCT	CTCTTCAATA	TCTATGTTTT
601	GATGGTAATT	TATCTCAGAT	TCAGTCACAA	GGCCAGCTTT	CCTCCTTTTC
651	TCACCTCACT	TCGCTTCAAA	CTCTACAAAT	CCGTAATCTC	CAATCACTTG
701	CTGCATTAGC	ACTGCCCTCC	TCCCTCTCTC	ACCTGACCAT	CCTCAATTTT
751	CCTAATCTCC	AATCACTTTC	AGAATCAGCA	CTGCCCTCCT	CCCTCTCTCA
801	CCTGATCATA	GATGATTGCC	CTAATCTCCA	ATCACTTTCA	GAATCAGCAC
851	TGCCCTCCTC	CCTCTCTCAC	CTGGACATCT	CCAATTGcCC	TAATCTCCAA
901	TCACTTTTCA	AATCAGCACT	GCCCTCCTCC	CTCTCTAGCC	TGACCATCTA
951	TGATTGCCCT	AATCTCCAAT	CACTtCCAGT	AAAAGGGATG	CGTCTTCCC
1001	TCTCTGAACT	AGCAATTTCC	AAATGTCCAT	TGCTCAAACC	ACTACTAGAA
1051	TTTGAAAGG	GGGAATACTG	GCCAAATATT	GCTCATATCC	CCTCCATATA
1101	CATCGATTGG	GAACGCATGT	AATGATTAAA	ACGAATGGCT	CCCCAACTGA
1151	TATGTGGATT	TTGAAGAGCG	AGTACGACAA	GTCTGGTACA	TCAATTGTCC
1201	GTAGGAAGTG	TTTCTAAGTG	AATTTTCAGG	TTTGTGTGTA	TAGGCAAGTC
1251	TTTGAGATGC	GACTATCAAA	GAAGGGCGAT	TACGATCAGT	GTACCGCTGA
1301	TATTATTTCA	TGTTTCCAGT	GCAAGCTTCT	TTTGTAAGTT	GACAAACTTG
1351	ATTAGTTCTC	GTGCCGAATT	C		



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## FIG. 7

1	CCAGGGTTTT	CCCAGTCACG	ACGTTGTAAA	ACGACGGCCA	GTGAATTGTA
51	ATACGACTCA	CTATAGGGCG	AATTGGAGCT	CCACCGCGGT	GGCGGcCGCT
101	CTAGAACTAG	TGGATcCCCC	CGGGCTGCAG	GAATTCTATG	GCAGATTGTC
151	CTCCAAAAAG	CCTTTTAACT	GTCTTGAGAA	GCTTGAATTT	GAAGATATGA
201	CGGGGTGGAA	GCAATGGCAC	GCACTAGGAA	TGGAGAGTT	CCCTACACTT
251	GAGAACCCTTT	CCATTAAAAA	TTGCCCTGAG	CTCAGTTTGA	AGATACCCAT
301	CCAATTTTCA	AGTTTAAAAA	GGTTACAAGT	TAGAGGTTGT	CCAGTTGTTT
351	TtGATGATGC	TCAACTGTTT	AGATCCCAAC	TTGAAGCAAT	GAAGCAGATT
401	GAAGCATTAt	AtATACGTGA	TTGTAACTCT	ATTACCTCCT	TTCCTTTTAG
451	CATACTGCCA	ACTACCTTGA	AGACAATAGA	GATATCTGGT	TGCCCCAAAT
501	TGAAATTGGA	GGCGCCAGTT	GGTGAGATGT	TTGTGGAGTa	TTTGAGTGTG
551	ATTGATTGTG	GTTGTGTAGA	TGATAATATC	ATTAGAGTTT	CTCCCAGCAG
601	CGTGTAATTT	GAGTATTATG	AGTTGCCACA	ACTTTACTAG	GTTTTtGATT
651	CCTACTGCAA	CTGAAACTCT	CACTATTTCT	AATTGTGAGA	ATGTTGAAAA
701	ACTATCGGTG	GCACTGTGGAG	GAGCGGCCCA	GATGACGTTA	CTGCATaTTT
751	tGAAGTGTA	GAAGCTCAAG	TGTCTGCCAG	AACGTATGCA	GGAACCTCCTT
801	CCATCTCTCA	AGGATTTGTA	TCTTTCCAAT	TGTCCAGAAA	TAGAAGGAGA
851	ATTGCCCTTC	AATTTACATA	AACTCOGTAT	CAGTGATTGC	AAGAAACTGG
901	TGAATGGCCG	AAAGGAGTGG	CATTTACAGA	GACTCACAGA	GTTAGTGATC
951	CATCATGATG	GGAGTGACGA	AGATATTGAA	CATTGGGAGT	TGCCCTGTTC
1001	TATTACAGAA	CTTGAGGgTA	TACAATATGA	TAACATTAAG	CAGCCAACAT
1051	CTCAAAAGCC	TCACCTCTCT	TCAATGTCTA	AGTATTGGTG	GTAATTTATC
1101	TCAGATTGGC	CGTCTTTCCT	CCTTTTCTCA	CCTCACTTCG	CTTCAAACTC
1151	TACAAATCAG	GAATTTGGGT	AATCTCCAAT	CACCTGCTGA	ATCAGCACTG
1201	CCATCCTCCC	TCTCTCACCT	GACCATCTCC	CGTTGCCCGA	ATCTCCAATC
1251	ACTTGCTGAA	TCAGCACTGC	CCTCCTCCCT	CTCTCACCTG	AACATCTATG
1301	ATTGCCCGAA	TCTCCAATTA	CTACCTGAAT	CAGCACTGCC	CTCCTCCCTC
1351	TCTCACCTGG	ACATCTCCCA	TTGTCCTAAT	CTCCAATCAC	TACCTGAATC
1401	AGCACTGCTC	TCCTCCCTCT	CTCACCTGGA	CATCTCCAC	TGTCCTAATC
1451	TCCAATCACT	TGCTGAATCA	GCACTGCCCT	CCTCCCTCTC	TCACCTGACC
1501	ATCTCCCAT	GCCCTAATCT	CCATTCACTT	TCAGAAAAAG	GGATGCCCTC
1551	TTCCCTCTCT	AAACTATCTA	TTTCCAAATG	TTCATTGCTC	ACACCACTAC
1601	TAGAATTTAA	CAAGGGGGAA	TACTGGACAA	ATATTGCTCA	TATCTCCACC
1651	ATACAGATCG	ATTGGAAATG	CATGTAATGA	TTAAAACGAA	TGACTCCCCA
1701	ACTGATATGT	GGATTTAGAA	GAGCGAGTaC	GACAAGTCTG	GTACATCAAT
1751	TGTCCGTAGG	AAGTGTTFCT	AAGTGAATTT	TCAGGTCTGT	TGTTATAGGC
1801	AAGTCTTTGA	GATGTGACTA	TCAAAGAAGG	GCGATTACAA	TCAGTGTACC
1851	GCTGATACTA	TTTCATGTTT	CCAGTGCTAC	AGTGCAAGCC	TCTTTTGTA
1901	GTTGnCAAC	TCGATTAGTT	AATATGTTTG	GGACTCAACT	ACTACTCATT
1951	TTGTAAGACT	TAAGTACAGA	AAATCAAATT	AGAATTATAA	CTCGCGATGG
2001	TTGAGTAAAC	TCCAAGAAGC	TCGTGCC		

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I2C-1 .....MEIGLAIGGAFLLSSALNVLFDR LAPNGDLLNMFRKH 36  
 I2C-2 .....V 36  
 RPM1 .....MAS TVDFGIGRILSV ENETL SGVHGE 30  
 RPS2 ..... 0  
 N-GENE .....MASSSSSSRWYS VFLS GE 21  
 L6 MSYLREVATAVALLLPFILLNKFWRPNKDSIVNDDDDSTSEVDI DST PSGSPFSVEYEVFLS GP 70  
 CON.

I2C-1 TDDVELFEKLGDIILSLQI.VLSDAENKKASHQFVSQWLHKLQTAVDAAENLIEQVN..YEALRLKVETS 103  
 I2C-2 K H K LK KMT RGI . Q PS RD NE RD S E .. GQ 103  
 RPM1 I ..... MKKE IMKS.F E T.H HGG GSTTTTQLF F ANTRD AY IEDILDEPGYHIBGY 92  
 RPS2 .....MDFISSLIVGCAQ. CESH MAEKRGHKT.... RQ ITDL TA GDLKAIRDD T RIQ.. 57  
 N-GENE DTRKTFTSH YEV NDRG KTFQ DXRLEYGATIPGELCKAIESQF IVVFS NYATSRWC NEL KIM 91  
 L6 DTREQFTDF YQS RRYK HTFR DDELLKGKEIGPNL RAIDQSKIYVPIISSGYADSKWC MELA IV 140  
 CON.

I2C-1 NQOVSDL.NLCLSDDFFLNIKKLEDTIKKLEVLEKQIGRLGLXEPIS.....TK..... 153  
 I2C-2 H NF ETS QOV E D ET KD QE L Y D ..... 154  
 RPM1 RSCAKIWRAPHFPR..YMWARHSLAQKLGVMN MIQS SD.SM RYH .....ENYQAALLPPID 150  
 RPS2 QDGLEGRSCSNRAREWLSAVQVTETK ALL VRFRREQ TRMRRYL CPGCADIYLC KVSAILKSIG 127  
 N-GENE ECK.TRFKQTVIPIFYDVDP SHVRNOKESFAKAF EHETKYKDDVEG QRWRIALNEAANLKSGCDNRDK 160  
 L6 RR EE PRRII PIFYMVDPSDVRBQ GCYKAFR HANKF..DGQT QNWKDALKKVGDLKGWHIGKND 208  
 CON.

I  
P-LOOP

I2C-1 .....QETRTP.STSLVDDSGIFGRKNEIEN..LVGRLLS.MDTRKKNLAVVPIVGHGGMGKTTLAKAV 213  
 I2C-2 .....L . I EPD QS D.. ID .EGASG T L 214  
 RPM1 DGDAKWVNNISE. SLFFSENSLV IDAPKGR.. I .PEPQ ...I AV S SANI 213  
 RPS2 ELRERSEAIK D.GG IQVTCREIPI SVVG TIMMEQV E.FLSEEEERGIIGVY P V MQSI 195  
 N-GENE TDADCIQIVDQI SK CXI LSY.LQ IVGIDTHLEKIE LLEIGINGVRIMG W V I R I 229  
 L6 KQCAIADKVSADIWSHISKENL LETDELVG DDHITAV EKSLDSE VTM GLY I T 278  
 CON.

αα GMGGαGKTT

I2C-1 YNDERVQKH.FGLTAWFCVSEAYDAFRITKGLLQEI.GSTDLKADDNLNLQVVKLADDNLNLQVVKLKE 281  
 I2C-2 S KN . D K N . I .....V 278  
 RPM1 FKSQS KR . ESY VTI KS VIEDVFRTHIK FYKEA TQIPAE YS .....GYRE VE V 274  
 RPS2 N ELITKG QYDVLI VQM REFGECT QAVGARGLG W E ETGENRA .....IYR 250  
 N-GENE FDTLLGR.....MDSSYQFDG CFLXD KE..NKRGNH LQALLSE LR...EKANYN EEDGKHOMAS 289  
 L6 KI.....SSC.FDCCCFIDN RETQEKDGVVVLQK LVSEILRIDSGSVGPN DSGGRKTI 337  
 CON.

II III

I2C-1 KLNGKRFLVLDVWVNDNYPEWDDLRLNLFLOQDIGSKIIVTRKESVALM...MDSGAIYMGILSSSEDSW 348  
 I2C-2 R KE K I N E V V D ... GNEQ S N T A 335  
 RPM1 Y QS YI TTGL.. REISIALPD IY RVMM DMN SPYIGIG TKHEIEL KEDEA 342  
 RPS2 A RQ LL EEIDL KTGVP..PDRENKC VMF .. I CNNMGAEYKLRVEF EKKHA 315  
 N-GENE R RS KV I I.DNKDHYLEY AGGLEWFGN R I DKHLI....EKNDIYEYTA PDHE I 354  
 L6 RVSRFKI .DEKFKFE M GSPKDFISQ. RF I S SMF LGTLNENQCKLYEV SM KPR L 405  
 CON. K+αLαVLDDV S+ααTTR L

IV

I2C-1 ALFKRHSI.EHKDPKEHPEFEVGRQLADKCKGLPLALKALAGMLRSKSEVDEWRNLRSEIWELPSCSN 417  
 I2C-2 S Q AF. NM MG S L R A T E KC R..D 402  
 RPM1 V SNKAPPASLEQCRTQNL PIARKLVER O IAS GS MST KFES KKVYSTLN NNNHE 412  
 RPS2 E CSKVM..R LL SSSIRRLAEI VS G IT G AMAHRETEE IHASEVLTRFPAEMKG 384  
 N-GENE Q Q AFGKE...VPNEN KLSLEVNYA VWGSL .HNLRLT KSAIE....HMKN.S 415  
 L6 E SK AFKKN...TPPSYY TLANDVV TTA T VIGSL .F Q IAV EDT E....Q RRTL 467  
 CON. LF GLPLAL BW

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V

I2C-1 ..GILPALMLSYNDLPAH.LKQCPAYCAIYPKDYQFPKEQVIHLWIANGLVHQFHS.....GNQYFIEL 478  
I2C-2 D . R SF F P PVEDEI IQDL F L 468  
RPM1 LKIVRSIMP F YP. R L SLP VN RMKRKRL RM M QRF EPIRGVKAEEVADS LN 481  
RPS2 MNYVFAL KF DN ESDL RS L LF EEHSIEI LVEY VGE FLTSS GVNTIYK YFLIGD 454  
N-GENE YS IDK KI DG EPKQQEMFLDIACFLRGE...E DYILQILESCHIGAERYG.....LRI 471  
L6 LDEVYDR KI DA NPEAKEIFLDIACFFIGQ...N EPIYM TDCNFYPASN.....I F 523  
CON. α L αSY L

VI

I2C-1 RSRSLFEMASEPSERDVEE.FLMHDLVNDLAQ.....IASSNHCIRLEDNKGSHMLEQC 531  
I2C-2 S RVPN GNIK L .....L KL ESQ 522  
RPM1 VY NMLQVILWNPFGRPKA. K VIWEI .....SVS 515  
RPS2 KAAC L .....TG EKTQVK NV RSF .....LWMASEQ TYKELIL 494  
N-GENE IDK VFI.....SEYNQVQ IQ MGKIYVNFQKD PGRSRLWLAKVEEVMST T TMAM AI 533  
L6 IQ CHIQV.....GDDDE K QLR MGREIVRREMLPWKRSRIWSAEBGIDL LNK SKVKAI 584  
CON. α MHDαα -

I2C-1 RHMSYSIGQDGEFEKLKSLFKSEQLRTLLPI..DIQPHYSKKLSKRVLHNILPTLRSLRALSLSHYQIEV 599  
I2C-2 L M Y G TP Y L TCSSVNYF .NP T KM E 541  
RPM1 KLERPCDVYNDSD.....GDDAAETMENYGRHLCIQ EMTPSIRA..TN H LVC SAKHKM L 576  
RPS2 VEP MGHTAPKA NWRQALVISL DNRIQT.....PEKLICPK T MLOQNS KK..... 548  
N-GENE MVS STLRFSNQAV NMKRLRVFNM.....GRSSTHYAIDYLPNN CFVCTNYPW... S 588  
L6 SI.PWGKYEFKS CFLN SELRY HA.....REAMLTGDFN L N KW ELPPYK GEDDP 643  
CON.

I2C-1 LPNDLFIKLLRFLDLSETSIKLPDSIFVLNLETLLSSCEYLEELPLQMEKLINLRHLDISNTRRL 669  
I2C-2 I R N KR C K. WH 660  
RPM1 S..... N A EDS S CLVTMF KY N KTQ.VK KNPH V ET NTKHSKIE 640  
RPS2 I TGF MEMPV V F EI L KY VE YH SM GTK.ISV QELGN RK K LQR QF 617  
N-GENE F STF..E M VH Q RH.....SLRH WTETKH PS ..... RI L WSK 632  
L6 PLTNY..TM N IIVI EHSR ADD.....WCGWRHMMKMA R KVV R ASNYSLYG RVRL DCW F 705  
CON.

I2C-1 KM..PLHLSRLKSLQVLVGAKFLVGWRMEYLGEAHNLYGSLSILELENNVDRREAVKAKMREKNHVEQL 737  
I2C-2 .. V D Q VVK P 728  
RPM1 EL.. GMWK K .....RY ITR N GHDSNW Y.....LGT .. VP IWQ..... 682  
RPS2 QTIPRDAICW SK E .NLYYSTA ELQSP DEABELGFAD YLENLTTLGITVLSL..... 677  
N-GENE T. RTPDFTCMPN EY..VN.. YQCSNL EVHHSLGCCSKVIG Y NDCKSLKRFPVCNVESLEYLGLR 696  
L6 P. KSIEVLSMTAIEHDEVD..IGELKKLKT VLRFCPIQKI GGTFGMLKGL L.....CL FN 762  
CON.

I2C-1 SLEWSESISADNSQTERDILDELPHKNIK.AVEITGYRGTNFPNWWADPLFVK.LVHLYLRNCKDCYSL 805  
I2C-2 Q.E K I L . K S 776  
RPM1 .....L .DLQVMDC.....FN EDELI .....N 702  
RPS2 .....E LKTLFEPGAL H Q.HLHVEECNELLYF LPSLTNHGRN RR SIKS H LEY 733  
N-GENE CDSL KLPEIYGRMKP.....E QIHMQGS I ELP.SSIFQYKTH TX LLWNM....KNLVA 751  
L6 WGTNLREVV IG LSSLKVLKTTGA EVEINEFPL LKELSTSSRIPNLSQLLD EV KVID GFDM 832  
CON.

I2C-1 PALGQ.....LPCLEFLSIRGMHGIRVVTEEFYG.....RLS 837  
I2C-2 ..... K VK ..... 828  
RPM1 GCMT ..... TRISLMV RE RDLC..... 725  
RPS2 VTPADFENDW S V TLHSL NLTR.....WG 763  
N-GENE LPSSICRLKS VS SVSGCSKLESLEPPEIGDLNLRVFDASDTLILRP.....P 801  
L6 PASPSEDESSVWVKV...SKLKSQLEKTRINNVVDDASSGGHLPRYLLPTSILTYLKIYQCTEPTW P 899  
CON.

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I2C-1 SKKPFNSLVKLRFEMPEWKQWHTLG.IGEFPTLEKLSIKNC.....PELSLEIPIQFSSLKRLD.... 896  
 I2C-2 C E E T A . I ..... FRVFGC 891  
 RPM1 ..DSL KIKRI LSLSIDEKEP E. DDL..... 753  
 RPS2 NSVSQDC RNI CINISHCNKLNVSQVQL K VIELFD .....R IKEL SEHE P..... 818  
 N-GENE IIRL K II M RGFKDGVHFEPPPPVAEGLHS Y NLSY NLID GG PE .GSL K L..S 866  
 L6 GIENLEN TS EVN IPQTLGGDLD L.QGLRS I R RKNVGLARIKG KDLLCSSTCK RKFYITEC 968  
 CON.

I2C-1 .....ICDCKSVTSFPPFSILPTTLKRIKISGCPKLKLEAPVGE..MFVEY 939  
 I2C-2 FVVF YDAQVLRSQLCEMKQIEEY R N T D C MS L E 960  
 RPM1 ..... ATASIE F AGXLERVPSWNT 767  
 RPS2 .....VEDPT FPS TLRTRDL E N.....SILPSR 847  
 N-GENE RNNFEHLPPSSIA QLGALGSLDLK QRL QL ..E PE NELHVDCHMA FIHYLVTKRKKLHR 930  
 L6 POLIELLPCELGVQTVVPSMAELT R PRL.EVG..PMIRS PKFPM..... KLDLAVANITKE D 1030  
 CON.

I2C-1 LSVIDCGCV.....DDISPEFLPTARQLSIENCHNVTRFLIPTATESLH.IRNC.. 987  
 I2C-2 F EE ..... E R G . T . EN 1006  
 RPM1 ..QNLTYL.....GLRGSQLOEN .I QTLPRLVWLSFYN YMGPR.L F... 821  
 RPS2 VKLD AENDTMYNLFAYTMFQNISSMR ISASDS SLTVFTGQPYPEKIPSWFHHQGD SVSVN.... 996  
 N-GENE DA GSLEELV SLELELDDTSSGIERIVS SKLQRLTTLVV.....K PSLREIEGL E KSLQDLYL 1093  
 L6  
 CON.

I2C-1 .EKLSMACGGAAQLTSLNINGCKKLKCLPELLPSLKEIRLTYCPE.....IEGELPPNLQILDIRYC 1048  
 I2C-2 V D S Q N ..... K Y D 1068  
 RPM1 .....QGQFN K LEIVQM H T VV..... DGAM E K YV A 860  
 RPS2 E WWK LEKDQPNEE C.....Y R FV N..... 909  
 N-GENE ... PENWYIPDKFLGFVAVCYRS IDTTAH IPVCDK.....MSRMTQK A....LSECDTE 1048  
 L6 EGCT LGRLPLEK KE D G PD TE VQTVVAVPS GLTIRDCPRLEVGPMIQS KFPMLNELTSL 1163  
 CON.

I2C-1 KKLVNGRKEW.HLQRLTELWIKHDGSDIEHIEWELP....SSIQRLEFIFNLKTLSSQHLKSLTSLQFLRI 1113  
 I2C-2 K V Y D ....C T EV I Y C 1133  
 RPM1 RG EYVPRGIEN IN Q HLI .V NQLV RIRGE....G VD .....SRV HIPAIKHYFR 914  
 RPS2 ..... 909  
 N-GENE SSNYSEWDIHFFVPPFAG DTSKANGKTPNDYGIIRLSF GEEKMYGLR LYKEGPEVNA LQRENSN 1118  
 L6 MWNITKED LEV GS E DSELETL DTCSSI RISF L KL K TTLIVEVP LREIEG AE KS 1232  
 CON.

I2C-1 VGNLSQFQSQGQLSSFSHLTSLQTLQIWNFLNLQSLPESALPSSLSHLIISNCPNLQSLPLKGMPPSSLST 1183  
 I2C-2 D PI I H S Q E FH N K 1203  
 RPM1 TD G FYV ... 926  
 RPS2 ..... 909  
 N-GENE EPTEHSTGIRR.TQYNNR FYE ....ING.....1144  
 L6 LYLEGCTSLERLWPDQQQ G KN NVLDIQGCK SVDH SALKTT PPRARITWPDQ YR.....1294  
 CON.

I2C-1 LSISKCPLLTPLLLEFDKGEYWTEIAHIPTIQIDECH 1220  
 I2C-2 L G PQ L W YI 1240  
 RPM1 ..... 926  
 RPS2 ..... 909  
 N-GENE ..... 1144  
 L6 ..... 1437  
 CON.



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FIG.10

I2C-1	EEFYGRLSSKKPFNSLVKLRFEDMPEWKQWHTLGIGEPPTLEKLSIKNCPELSLEIPIQF	889
I2C-2	C E E T A I	880
I2C-3	.....	
I2C-4	Q C E E TG A N K	60
CON.	EEFYGRLSSKKPFN L KL FEDM WKQWH LGIGEPPTLE LSI NCPLESL IPIQF	
I2C-1	SSLKRLD.....ICDCKSVTSFPFSILPTTLKRIKIS	921
I2C-2	FRVFGCPVVFYDAQVLRSQLBGMKQIEIY R N T D	940
I2C-3	.....N	1
I2C-4	QVRGCPVVFDDAQLFRSQLBGMKQIEALY R N I T E	120
CON.	SSLKR I DC S TSFPFSILPTTLK I I	
I2C-1	GCPKLKLEAPVGE..MFVEYLSVIDCGCVDDISPEFLPTARQLSIENCHNVTRFLIPTAT	979
I2C-2	C MS L EF EE ... E R G	996
I2C-3	SARE .. N E K I TD Q I	59
I2C-4	F .. L A CK MS F	178
CON.	LK EAPV E MFVE SV CGCVDDIS EFLP A L I C N TRFLIPTAT	
I2C-1	ESLHIRNC...EKLSMACGGAAQTLNINWGCKKLKCLP....ELLPSLKELRRLTYCPET	1032
I2C-2	T ENV D S .... Q N	1052
I2C-3	T T E ENV V M I SE ERMQ SD	119
I2C-4	T T S ENV V M L H LK ERMQ D Y SN	238
CON.	E L I NC EKLS ACGGAAQ T L I CKKLKCLP ELLPSLKE L CPET	
I2C-1	EGELPFNLQILDRIYCKKLNVGRKEWHLQRLTELWIKHGDGSDIEHIEWELPSSIQRLEFIF	1092
I2C-2	K Y D K V Y D C T EV	1112
I2C-3	K Y S H D S T C	179
I2C-4	HK R SD V H D C N RVY	298
CON.	EGELPFNL L I CKKLNVGRKEWHLQRLT L I HDGSD IEHWELP SI L	
I2C-1	NLKTLSQHLKSLTSLQFLRIVGNLSQFQSQGLSSPSHLTSLQTLQIWNF.....	1143
I2C-2	I Y C D PI I	1164
I2C-3	I Y C F D I R.....	228
I2C-4	MI C S G I... R R GNLSLAES	355
CON.	N TLSSQHLKSLTSLQ L GNLS QSQ LSSPSHLTSLQTLQI	
I2C-1	.....	1143
I2C-2	.....	1163
I2C-3	.....NLQSLAALALPSSLSHLTILNFPNLQSLSESALPSSLSHLIIDDC	273
I2C-4	ALPSSLSHLTISRCPNLQSLAESALPSSLSHLNIYDCPNLQLLPESALPSSLSHLIDISHC	415
CON.		
I2C-1	.....LNQSLPESALPSSLSHLIISNCPNLQSLPLKGMPS	1180
I2C-2	.....H S Q E FH N	1200
I2C-3	PNLQSLSESALPSSLSHLIDISNCP S S T YD V	333
I2C-4	PNLQSLPESALLSSLSHLIDISHCP A T H H SE	475
CON.	LNQSL ESALPSSLS L I CPNL SL GMPSS	
I2C-1	LSTLSISKCPLLTPLLEFDKGEYWTEIAHIPTIQIDEECH	1220
I2C-2	K L G PQ L W YI	1240
I2C-3	E A K G PN S Y W R	373
I2C-4	K S N N S WK	515
CON.	LS L IS C LL PLLEF KGEYW IAH I ID	

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FIG. 11

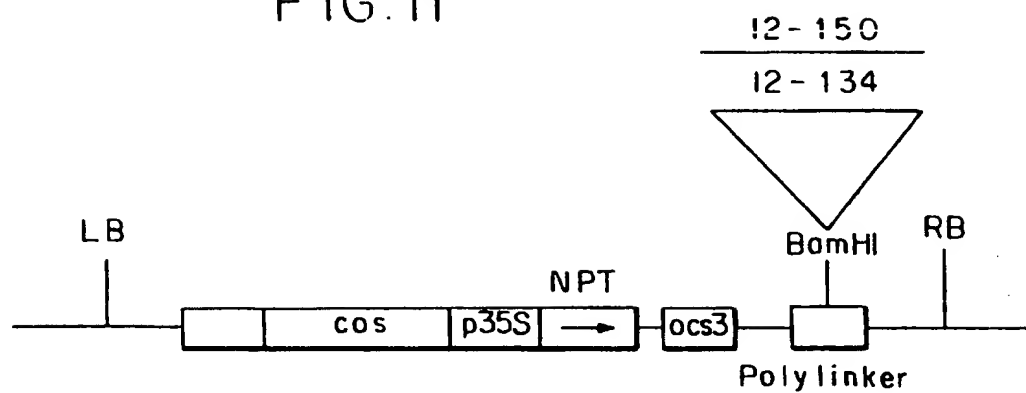


FIG. 12A

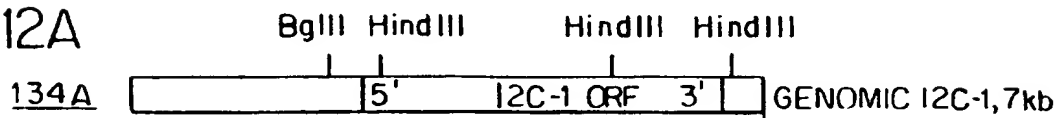


FIG. 12B

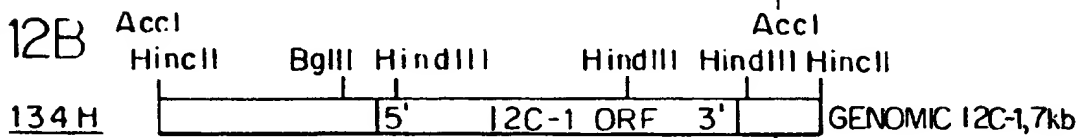


FIG. 12C

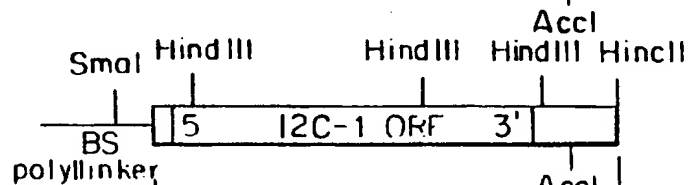
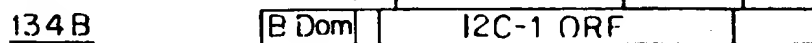
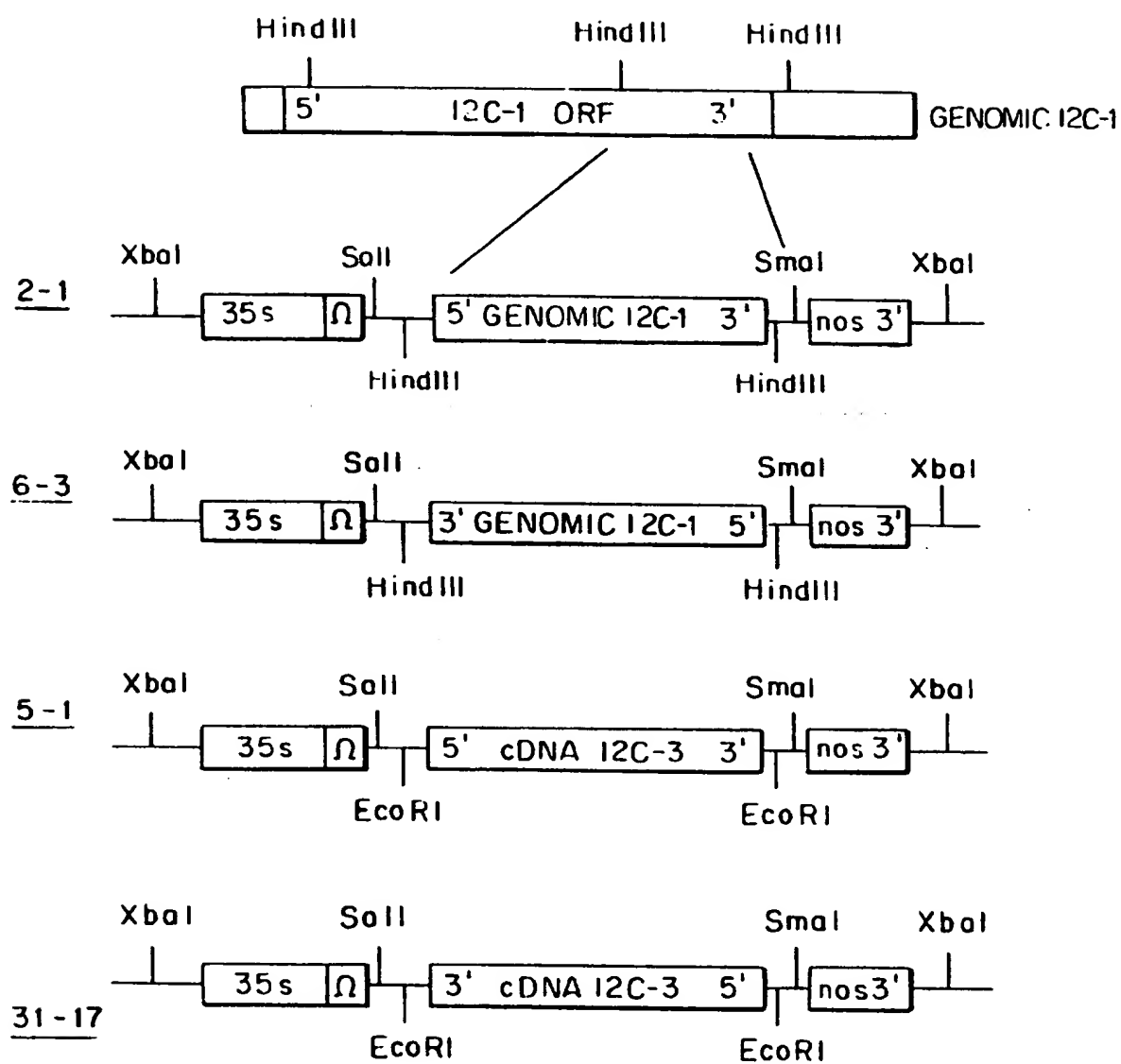


FIG. 12D



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FIG. 13





## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/05272

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01H 1/04, 5/00; C12N 5/04, 15/29, 15/64, 15/82; C12Q 1/00;

US CL : 800/205, DIG 44; 435/6, 240.4, 320.1; 47/58, DIG 1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/205; 435/240.4, 320.1; 47/58, DIG 1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SWISSPROT, MPSRCH

search terms: SEQ. ID NOS. 1-4

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y  Y, P	US, A, 5,530,187 (LAMB et al.) 25 June 1996 (25/06/96), see entire document, especially columns 2-3.	1 -- 1-9
	US, A, 5,437,697 (SEBASTIAN ET AL.) 01 August 1995 (01/08/95), see entire document, especially columns 36-37.	1,10-13
X -- Y	SARFATTI et al. RFLP mapping of I1, a new locus in tomato conferring resistance against Fusarium oxysporum f. sp. lycopersici race 1. Theor. Appl. Genet. 1991, Vol. 82, pages 22-26, especially 25.	1 ----- 1,10-13



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
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* L		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
* O	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* P	* A	document published prior to the international filing date but later than the priority date claimed
		document member of the same patent family

Date of the actual completion of the international search

14 AUGUST 1996

Date of mailing of the international search report

08 SEP 1996

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/05272

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOURNIVAL et al. An isozyme marker for resistance to race 3 of <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> in tomato. Theor. Appl. Genet. 1989, Vol. 78, pages 489-494, especially 493.	1,10-13
Y	BOURNIVAL et al. New sources of genetic resistance to race 3 of fusarium wilt of tomato. Plant Disease. 1991, Vol. 75, pages 281-284.	1,10-13
Y	US, A, 5,437,697 (SEBASTIAN ET AL.) 01 August 1995 (01/08/95), see entire document, especially columns 36-37.	1,10-13
Y, P	STASKAWICZ et al. Molecular genetics of plant disease resistance. Science. 05 May 1995, Vol. 268, pages 661-667, especially 666.	1,10-13